STUDIES ON TSUTSUGAMUSHI DISEASE

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STUDIES ON
TSUTSUGAMUSHI DISEASE

(JAPANESE FLOOD FEVER)

BY

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PREFATORY NOTE.

When the author of this monograph was in the United States, last spring, we learned that he desired to publish an English translation, and at our suggestion he submitted to us one prepared by two Japanese colleagues. It was found to be so interesting and complete, that we proposed publishing it as two special numbers of the BULLETIN. It is felt that the studies of Dr. Kawamura on tsutsugamushi disease should be made available to a larger circle of readers than would be possible were the text to remain in the original Japanese. Dr. Kawamura has authorized this translation, and the MEDICAL BULLETIN feels that it is rendering a real service to scientific medicine in publishing this work.

The translation was edited and carefully compared with the original Japanese edition, by Drs. Foot and Tashiro, and appears in this volume as they submitted it to us. We greatly regret that it was found advisable to delete a number of tables based upon the protocols of several of the experiments cited in the text, on account of the great expense that their publication would entail; but we feel that Dr. Kawamura has so fully and ably summarized each table, that little would be gained by publishing these unavoidably bulky documents in this volume.

N. C. FOOT,
ALFRED FRIEDLANDER.

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Figure 1
Endemic Localities of Tsutsugamushi Disease in Japanese Empire

STUDIES ON TSUTSUGAMUSHI DISEASE

INTRODUCTION

Tsutsugamushi disease occurs as a specific fever, distributed along the course of large rivers in Japan, and is found chiefly in the northern part of that country in the prefectures of Niigata, Akita and Yamagata. The first scientific clinical report of the disease was published in 1878 by Bälz and Kawakami (9) who studied it at Kurotsu, in the prefecture of Niigata, and named it "river" or "flood fever," since it occurs along rivers, usually in conjunction with floods.

In 1908 an endemic, exanthematous and bubonic disease appeared in the eastern part of Formosa, in 1914 it was identified as tsutsugamushi disease by Hatori (39, 40, 41) and in 1920 I showed that it was identical with the endemics occurring in northern Japan (73). (Fig. 1.) Outside of Japan Schuffner (164, 165, 166) has been investigating an endemic, closely resembling tsutsugamushi disease, at Deli, Sumatra, since 1902. He calls it "Deli pseudotyphoid," or "atypical tsutsugamushi disease." Other reports on febrile affections resembling tsutsugamushi have been published by Ashburn and Craig (8) (two cases in the Philippines), Noc and Gartron (one case in Saigon), and Dowden (24) (one case in the Malay Peninsula). In 1910 Smith found a tsutsugamushi-like endemic in Mossman, North Queensland; Breinl, Priestly, and Fielding (18) also reporting on it. The investigation of a similar disease in the South Sea Islands has been unsatisfactory, and it would be unwise to identify it with tsutsugamushi disease without further proof.

Historical: A Chinese medical book written in the Sei era, in the sixth century, mentions tsutsugamushi as a "sand-louse." The insect* is described in detail in "Honso

* Instead of "acarid" we shall use the popular term "insect" for the sake of convenience in connection with this mite.

Komoku," by Lishiting, written in the sixteenth century. He says: "The sand-louse is found along river banks during the summer. It is tiny, not as easily discerned as the tip of a hair, and is red. If one walk along the river bank, or through weeds, or bathe in the water in the morning or evening, after a rain, the mites will attach themselves to one's skin and bore into it. It becomes reddened, and if one rub the reddened area with the hand, there is sharp pain, like the pricking of a needle. Within three days all the joints become painful, chills and fever appear, and pox form where the skin was red. When these ripen they are scraped with a bamboo stick or, if deep, with a needle to remove the mites. If these be placed upon a finger nail and observed in strong light, their movements may be readily perceived. If the mite be too deeply embedded in the skin to be removed in this way, burning joss sticks may be employed to kill it," etc. The disease was, no doubt, in existence in South China, along the banks of the Yong Tsuching river in Changsha, Yuan, Kiang, and Kiaichow provinces; but to-day it is not found in China.

Genkei Ohtomo describes the tsutsugamushi in 1819 as a hair- or sand-louse, occurring in Akita prefecture, and found it to be the same as that of southern China. The earliest description of the disease in Niigata prefecture was written by Hakuju Hashimoto, in 1810. He described it as the "tsutsuga," found along the banks of the upper tributaries of the Shinano river. In Yamagata prefecture, the disease was found along the upper waters of the Mogami river in 1835, but it gradually decreased in incidence when the river was widened and an embankment built to prevent floods, and had disappeared entirely about fifty-five years ago, at the beginning of the Meiji era. Since 1904 it has reappeared there and was identified in 1913 as tsutsugamushi disease.

The origin of the word "tsutsugamushi" is obscure; according to an old Chinese book it has two meanings: "poisonous worm" and "wild beast." At any rate, it denotes a noxious living organism that may injure or kill mankind. Nowadays the meaning has been shifted to indicate "disease" or "grief."

Distribution: Tsutsugamushi disease is found in fairly definitely limited localities in the flood basins of the large

Figure 2
Endemic Localities of Tsutsugamushi Disease in Niigata Prefecture

rivers. In Niigata prefecture there are three localities along the Shinano and Akano rivers; in Akita prefecture one along the Omono river; and in Yamagata prefecture one along the Mogami river. While the disease occurs in the flood districts of certain rivers, it does not in those of others. It is found chiefly in Niigata prefecture. In Formosa it occurs not only along the rivers, but also in forest and reclaimed land. (Fig. 2.)

Investigations on Tsutsugamushi disease in Japan: Many investigators have studied tsutsugamushi disease in Japan since Bälz's time, the object of these studies usually being to discover its etiology. Since Kitasata (78, 79) worked on it in 1893, Asakawa, Kitashima (80), Miyajima (81, 84, 103-109) (all members of his institute); Ogata (134-144) and Ishiwara (59) of the Imperial University; and Hayashi (42-45) of Aichi Medical College, have devoted much energy to its investigation. The reports of the Tsutsugamushi Disease Investigation Committee of the Hokuetsu Medical Society (58) are worthy of special mention. Tanaka (172-175) has been studying the disease since 1892, in Akita prefecture, not only from the etiological standpoint, but also from its clinical and pathological aspects. Not only has its etiology not been clearly established, but no complete clinical observations have been carried out according to modern methods, the pathology of the disease has been obscure because of the difficulty encountered in obtaining permissions for necropsies and practically nothing has been discovered for its prevention and cure. Thus the study of this disease has been incomplete in every respect.

I (63-74) have been investigating it under the auspices of various private and public organizations ever since I came to Niigata Medical College in 1913, the members of my laboratory, particularly Hattori, Yamaguchi, Sekimoto, and Ohmori, and many students and nurses assisting me. Drs. Arima and Sakai of the Tokyo Imperial University aided me in clinical study during 1913 and 1914 respectively. Throughout the first five years, the investigations were carried out for several weeks in each year, when the disease was most prevalent, at the endemic locality; since 1917 they have been continued in my laboratory throughout the entire year. Thus, for the past eleven years, we have been engaged in studying this disease

in all its aspects, and I believe that we have made definite progress except as regards its etiology. The following report includes the results of these investigations, some of which have already appeared in various journals, and it is needless to say that it embodies data obtained by my collaborators as well as by me. The success of this work is largely due to the material and moral assistance generously given us by various persons and organizations, and I feel that in publishing this report we are, in a measure, lightening our burden of indebtedness to them.

Let us here express our appreciation of the constant stimulus afforded us by many other investigators: Ogata, and Ishiwara, of Tokyo Imperial University; Kitajima, Miyajima, and Okumura of the Kitasato Institute; Hayashi of Aichi Medical College; Nagayo and his collaborators Miyagawa, Tamiya, Imamura, Mitamura, and Sato of the Government Institute of Infectious Diseases; all these have been indefatigable investigators of tsutsugamushi disease. Their work has, however, been limited to its etiology and pathogenesis.

CHAPTER I.

CLINICAL STUDIES OF TSUTSUGAMUSHI DISEASE.

Clinical reports have been made by Bälz, Tanaka, and by the Tsutsugamushi Investigation Committee of the Hokuetsu Medical Society. The following chapter is based upon the observation of ninety patients, most of them treated in the Investigation Station, a few cases in our Medical College Hospital. The number of patients and the dates of observation follow.

Year	No. of patients
1913	14
1914	18
1915	20
1916	13
1917	18
1918	1
1919	2
1920	2
1921	1
1922	1

The age and sex of these patients were as follows:

Age	Male	Female	Total
1 - 5	1	1	2
6 - 10	3	1	4
11 - 15	8	4	12
16 - 20	10	1	11
21 - 30	16	9	25
31 - 40	6	5	11
41 - 50	9	6	15
51 - 60	5	1	6
61 - 70	3	1	4
Total	61	29	90

There were four pregnant women aged respectively, 23, 25, 27, and 35. Of the ninety cases, seventy-eight represented the first, ten the second, and two the third attack.

1. Incubation Period.

As Bälz and Tanaka have said, it is difficult to determine the incubation period of this disease. Bälz estimates it at 4 to 7 days, Kitasato at 3 to 7, while Tanaka finds that it varies from 7 to 12 days. According to Oda's observation of fourteen

patients whose contacts could be traced definitely, the incubation period varies from 7 to 12 days, is frequently 8 days, and averages 9. Bälz and Kitasato probably estimated this period as beginning at the time of lymphnode enlargement and tenderness, which would account for this discrepancy; for the true onset is marked by general subjective symptoms which appear a few days later. Lymphatic tenderness may be elicited two days after the insect-bite, according to Tanaka and the Committee of Investigation; we, too, have observed two cases in which lymphatic tenderness came on two or three days before the onset of general symptoms.

If the time intervening between the bite and the onset of general symptoms be considered as the stage of incubation, there are twenty-six out of ninety cases whose incubation period may be reckoned as follows:

Incubation period	No. of cases
5 days	1
6 days	4
7 days	5
8 days	3
9 days	4
10 days	3
11 days	4
12 days	1
13 days	1

The average is 8.2 days, the extremes are 5 and 13 days, while the usual period is 6 to 11 days.

2. Prodromal Symptoms.

In the majority of cases there are no prodromata, in some there is general malaise, anorexia, headache, dizziness, nausea and photopsia. We have questioned several hundred people who have been bitten by the akamushi, the mite which transmits the disease, and find that many of them complain of headache, oppression, general malaise, numbness at the site of the bite, and often anorexia, shivering, or even chills and fever. No doubt these symptoms have nothing to do with tsutsugamushi disease, being caused by the secretions of the mite. These symptoms are transitory and subside in two or three days. Tanaka was able to elicit similar phenomena by injecting freshly macerated akamushi into volunteers. Three hours after such an injection there was flushing, a

sense of fear, languor, and a slight rise in temperature, but there was no typical local change at the site of the injection, nor tenderness in the lymphnodes.

These sensory disturbances may be overlooked unless the patient be naturally sensitive and observing; the bite of the akamushi is often first disclosed by a prickling sensation when the clothing, or the hand, is rubbed over it. Among our ninety patients there were about fifteen who noticed headache, lassitude, etc. at the time they were bitten. Thus, these are not prodromal symptoms, but are caused by the bite of the insect.

3. Symptoms of Tsutsugamushi Disease.

The typical symptoms in the early stages of the disease are general malaise, headache, a feeling of oppression, anorexia, insomnia, and photopsia; occasionally there is slight xanthopsia, tinnitus aurium, dizziness, pain in the joints, and epistaxis. Hot flushes and chills may occur and there may be constipation or, rarely, diarrhea. The shivering reported by previous investigators occurred in none of our cases.

As to objective symptoms; besides fever there is tenderness and swelling of regional lymphnodes, near which one may find flat, yellowish-brown, necrotic placques of skin surmounted by crusts. As Bälz and Tanaka have noted, skin necroses and tenderness over the regional lymphnodes are pathognomonic of this disease. In reinfected cases the changes at the site of the bite are often vague, but tender lymphnodes are always present.

A. Clinical Description of Cases.

a. The Abortive Case.

There were nine cases of this type: two 15 years old, four 15-20, and three 21-30. Typical changes at the site of the bite and slight swelling of the regional nodes, with slight tenderness, were noted. There was very slight fever, either continuous or remittent in type, lasting two to four days and ranging from 37.2° - 38.7° C. Occasionally there was no fever. There was but one case with a slight eruption which disappeared after two days. There were no other marked symptoms.

b. Typical Cases.

There were fifty-seven patients with typical symptoms. Before considering general symptoms, descriptions of mild, medium, and severe cases will be given.

i. Mild Case, male, 23, farmer. (Fig. 3.)

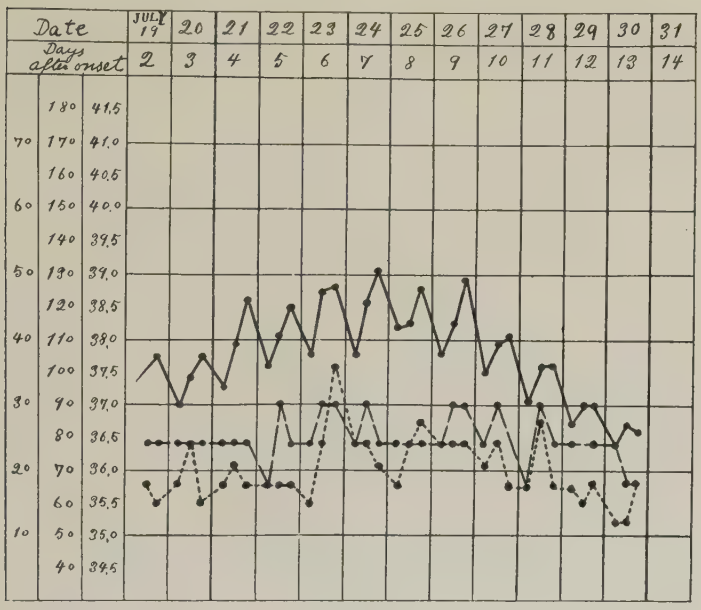


Figure 3

History: The patient had been bitten by the akamushi on several previous occasions, but had never had tsutsugamushi disease. Entering the endemic locality on July 7th, 1917, he was bitten in three places by akamushi, developed general lassitude on the 18th., and entered the hospital on the following day.

Physical Examination: Physical development fair. Facial expression normal. There is slight congestion of the conjunctivae, the tongue is moist and slightly coated, the throat somewhat congested. Gums red but not swollen, nasal mucosa slightly congested. The right margin of the heart is at the midsternal line, its upper margin at the second rib, its apex in the fourth space within the nipple line. There is a blowing murmur at the apex. Lungs normal. The liver is not swollen, there is no abdominal tenderness, and the spleen is not palpable. The patellar reflex is a little accentuated. Skin moist and warm. Pulse 62, expansile. No eruption noted.

The cervical nodes are somewhat enlarged and soft, the right axillary nodes average 2 to 3 cm. and are rather firm, the left are flat and soft. The right inguinal nodes are a few mm. in diameter, the left 1.5 x 3 cm. and tender. The right popliteal group is hard but not

tender, the left averages 1.5 cm. in diameter and is soft. An elevated, red area presents at the left margin of the anus, where the mite has bitten the patient and at the center of this area is a small, dirty crust. There is neither induration, nor tenderness.

Course of the illness: July 20th. General condition good, physical examination unaltered.

July 21st. No eruption.

July 22nd. Profuse sweating, patellar reflex increased, no eruption.

July 23rd. and 24th. Unchanged.

July 25th. Borborygmi palpable in abdomen, tenderness in umbilical region, no diarrhea, no eruption.

July 26th. General improvement, spleen hard and palpable.

July 27th. Fever has subsided, patient discharged.

ii. Moderately Severe Case. Male, 23, farmer. (Fig. 4.)

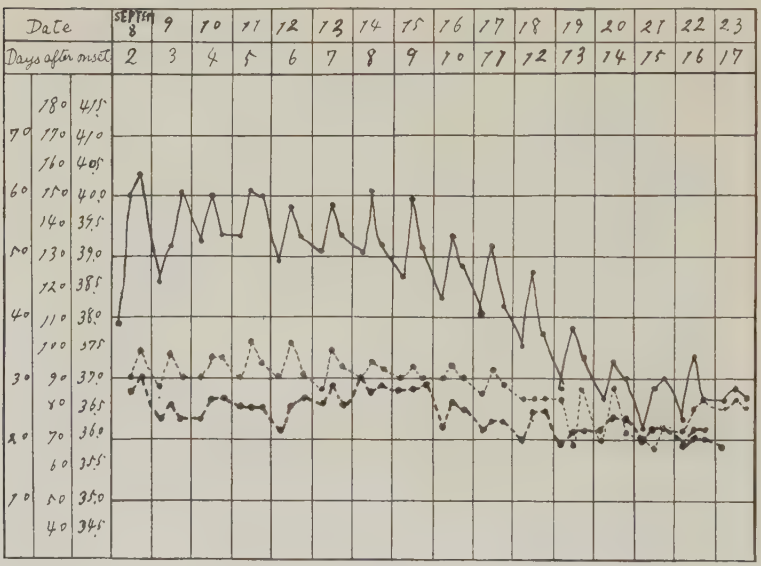


Figure 4
Moderately Severe Case. Male, 23 years old. Farmer

History: The patient has often been bitten by akamushi since childhood, but has never had tsutsugamushi disease. On September 7th, 1914, while carrying bundles of grass on his back, he felt prickling over the left scapula. There was no immediate bad effect. The next morning he had headache, lassitude and no appetite and remained in bed. He was not constipated. The left cervical nodes became painful and swollen that day, his temperature was 37.8° C. He entered the hospital on September 9th. Physical Examination: Physical condition and nourishment fair, expression normal, musculature well-developed, skin moist and feverish. The bite is seen in the left cervical region five cm. below the hair-margin, it is slightly elevated, 0.7 to 0.8 cm. in size, and red. At the center is a light brownish crust, about 0.5 cm.

in diameter. Supraclavicular lymphnodes swollen and somewhat tender; the submaxillary, axillary, and inguinal nodes are all swollen, hard, and tense.

Pulse large and expansile, regular, dicrotic; its rate is 84. The face is not flushed, the conjunctivae are somewhat congested; the nasal mucosa, while not congested, is covered by a yellowish secretion. The tongue is moist, grayish and coated. The gums are firm and there is neither congestion nor eruption over the palatal mucosa. The fauces are slightly congested, but the tonsils are not swollen.

The upper margin of the heart is at the upper border of the third rib, the left margin is in the nipple line. Heart-sounds pure. Lungs normal. Liver dullness: absolute, third intercostal space; relative, upper border of third rib. Liver and spleen not palpable, no abdominal tenderness present.

There is no eruption on the face, but a few questionable areas are seen on the flexor side of the forearms. There are several areas of papular eruption on the back, the flexor surface of the thighs, and the tibial side of the legs. No eruption noted on feet or soles. The patellar reflex is weak and no Achilles reflex is present.

Course of the Illness: September 10th. An eruption appears on face, readily palpable and firm.

September 11th. Complains of headache and lassitude, appeite somewhat disturbed, pulse rapid and dicrotic, spleen still palpable, liver not felt, eruption on chest and thighs extending.

September 12th. Eruption on chest and abdomen extending, that on the back has faded somewhat. It is dark brown. Tongue heavily coated grayish-white. Patellar reflex exaggerated and Achilles reflex has now appeared. Regional lymphnodes still tender.

September 13th. General condition the same. Lips dry, gums spongy. Lymphnodes still palpable. Eruptions subsiding. No changes noted in lungs or heart. The second pulmonic sound is accentuated. Spleen and liver not felt. Patellar reflex almost absent, Achilles reflex elicited with difficulty.

September 14th. General malaise. Some deafness. Pulse large and dicrotic. Spleen not felt. Conjunctivae still congested. Tongue coated, gums spongy. Dry râles are now heard over the bases behind. Eruption has faded somewhat and persists as dark spots on the arms, chest, and abdomen, but is almost invisible over the back.

September 15th. General malaise and deafness still present, tongue coated and dry, mucosa of mouth and throat dry, but not congested. Heart-sounds somewhat rough at apex. Spleen and liver not palpable. The eruption has almost faded, excepting over the thighs, where it persists as dark spots. Lymphnodes still distinctly palpable.

September 16th. Still deaf. Tongue moist, tremulous and coated, pharyngeal mucosa not congested. Spleen not palpable, but the liver is felt in the midline. Eruption gone. Pulse large and dicrotic. Heart and lungs negative. Lymphnodes still palpable, the left cervical still somewhat tender. Edema of the soles of the feet now present.

September 17th. Tongue coated, dry and tremulous; pulse large, expansile, and dicrotic. Heart and lungs negative. Deafness persists. Edema of the soles has subsided. Patellar reflex now increased. No tenderness of regional nodes and they have become somewhat softer.

September 18th. General condition better. Sleeping better. Eyes slightly congested, tongue moist and tremulous and still coated. Pharyngeal mucosa not congested. Gums soft. Achilles reflex distinct.

September 19th. General condition improved, pulse still dicrotic, large and expansile. Conjunctivae still congested. Tongue tremulous and coated. No congestion of pharyngeal mucosa. Gums soft. Regional nodes softer, still average about 1.5 cm.

September 20th. Congestion of eyes has cleared up.
September 21st. Condition unchanged.
September 22nd. General condition better. Pulse still dicrotic large and regular. Tongue moist but no longer coated. No congestion of pharynx. Heart and lungs negative.
September 23rd. Pulse still dicrotic, patellar reflex exaggerated, regional nodes soft, other nodes elastic and soft. Patient discharged.

iii. Severe Case (Recovered), 20, male, farmer. (Fig. 5.)

History: The patient had never been bitten by akamushi, nor had he had tsutsugamushi disease. He entered the endemic district in July, 1923, but was not aware of having been bitten. On August 1st. he felt feverish, developed chills on the 2nd., and anorexia on the 4th. He had been constipated since the 1st. On July 31st. there was a small reddened swelling at the outer canthus of the right eye, and a sensation as of a foreign body in the eye. On August 2nd. he noticed tenderness and swelling of the pre-auricular nodes. He entered the hospital on the fifth.

Physical Examination: A well-developed young man, facial expression normal, skin slightly flushed over face, generally warm and moist. Musculature and fatty panniculus moderately well developed.

Temperature 39° C. pulse large and expansile, not dicrotic, rate 116.

At the outer canthus of the right eye there is a small, reddish swelling with slight desquamation; at its upper border there is a round, yellowish-brown crust about 3 mm. in diameter. The lesion is slightly tender. The right pre-auricular nodes average 1 cm. in diameter and are firm; no similar swelling on left side. Submaxillary nodes are enlarged. The right axillary nodes are 3 x 2 cm. in size, hard, but not tender, those in the left axilla are not swollen. The right inguinal nodes are slightly swollen, the left less so; both chains are firm, the left less than the right; neither is tender. The right femoral nodes are 5 cm. in largest diameter, and tender.

On the inner side of the middle toe there is a healed wound about 2 mm. across and a similar one between the first and second toes. Neither wound is tender. The patient says that these were inflicted two weeks ago and that two or three days later the right femoral nodes became swollen and tender, and have remained so.

The eyelids and conjunctivae are slightly congested, the tongue coated and grayish, the gums reddened, the pharyngeal mucosa congested. The heart and lungs are normal, the liver and spleen not felt, there is no abdominal tenderness or resistance. Patellar reflex present on both sides. Body weight: 55.5 kgm.

Course of Illness: August 6th. General condition good, face flushed. Insomnia. At the inner side of the forearms a pinkish, papular eruption appeared this morning; this afternoon there is an eruption, not yet papular, on the cheeks and forehead.

August 7th. General condition and sleeplessness improved. The tenderness of regional lymphnodes persists. Axillary nodes hard and slightly tender. A finely papular eruption covers both sides of the forearms, shoulders, chest, neck, and face. No enanthem.

August 8th. General condition good, did not sleep well. Cervical nodes are hard, both axillary groups contain nodes 1.05 cm. in diameter, no tenderness. Eruption generally increased. Pulse regular and bounding.

August 9th. Eruption fading. The breath-sounds at the bases are somewhat rough, but there is no dullness. Pulse the same. Regional nodes unchanged. Both patellar reflexes increased. Patient complains of precordial pain.

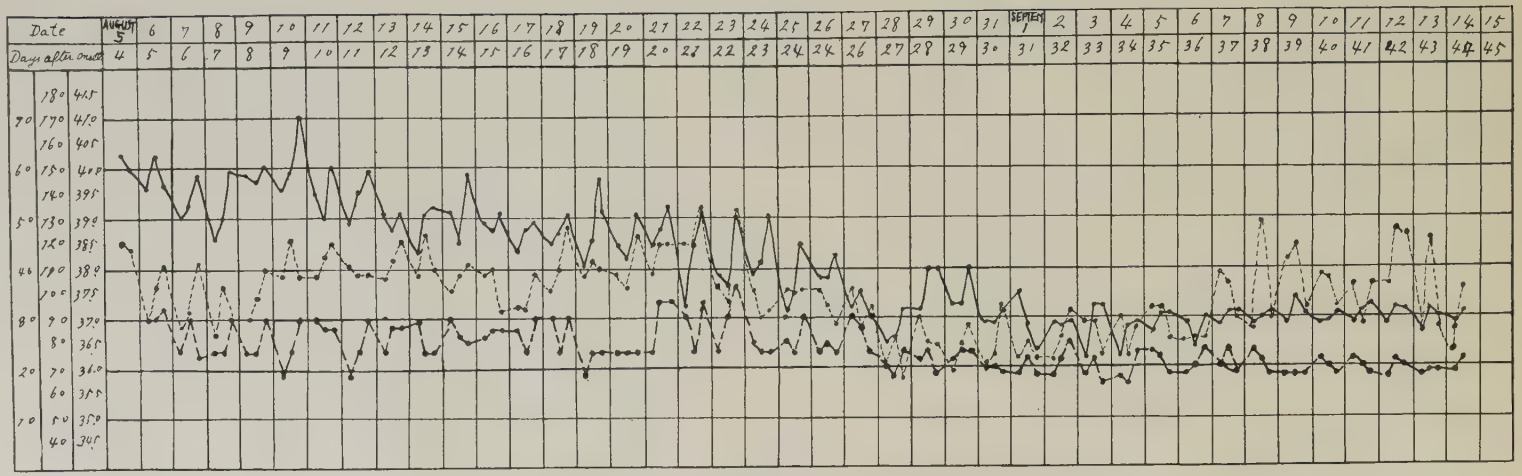

Date
AUGUST 5
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
SEPTEM 1
2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days after onset
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 24 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
180 41.5
170 41.0
160 40.5
150 40.0
140 39.5
130 39.0
120 38.5
110 38.0
100 37.5
90 37.0
80 36.5
70 36.0
60 35.5
50 35.0
40 34.5

Figure 5

August 10th. Sensations of oppression in the precordial region, but the pulse is regular and expansile. Both axillary groups and the gastrocnemius muscle are tender. Tinnitus aurium present. The patient complains of deafness. No sweating. Eruption on back has increased.

August 11th. The breathing in the left base is rough, no râles, slight dullness. Pulse regular, rather weak. Tenderness of both axillae persists. Patellar reflex increased. Eruption fading. Tenderness to pressure over the border of the tibia.

August 12th. Restless. Congestion of pharyngeal mucosa decreased. Pulse more rapid but strong and regular. Lungs normal. Eruption now represented by dark red spots. Liver felt a finger's breadth below the costal margin.

August 13th. Crusts have formed on a few of the discolored areas in the skin. The cervical, axillary, and inguinal nodes are tender. Tibial tenderness persists. Deafness has increased. Patellar reflex is exaggerated.

August 14th. Eruption almost gone. Lymphnodes remain tender and swollen. Deafness still increasing. Patellar reflex exaggerated.

August 15th. General condition better, both axillary groups tender, tibia the same, patellar reflexes +.

August 16th. The right epitrochlear nodes are now 1.5 cm. in diameter and hard. Patellar reflex +.

August 17th. Many miliaria crystallina have formed over the chest. Tibia and sternum both tender. Slight ankle clonus noted on the right.

August 18th. Continuous bleeding from the ear, where a small puncture was made yesterday afternoon for a blood-count. Insomnia. Appetite increased. Epistaxis this morning. Cervical and axillary nodes still tender. No change in chest. Pulse-rate increased, but beat is of good quality.

August 19th. In a somnolent condition. Miliaria increased. Slight ankle clonus on both sides. Moderate deafness.

August 20th. Somnolent all day. Tongue dry and clean, lips cracked. Pulse regular. Achilles reflex +. Tenderness over tibia and sternum persists. After removing the crust from the insect bite a round, clean ulcer is exposed 0.4 cm. in diameter; it is secreting profusely. Marked tremor of fingers.

August 21st. Slept poorly, appetite decreased. Mouth dry, lips more cracked. Pulse regular. Heart and lungs normal. Cervical nodes somewhat smaller and softer. Axillary nodes still tender. Ankle clonus and patellar reflex +.

August 22nd. Slept poorly. Pulse regular. Breath sounds, behind, are short but there are no râles. Tenderness over tibia less marked. Swelling of regional nodes distinctly decreased.

August 23rd. Tongue moist. Breath-sounds short and rough on both sides posteriorly. No tenderness, now, over tibia and strenum. Lymphnodes no longer tender. Patellar reflex still +.

August 24th. General condition poor. Delirious. Pulse strong and regular, with very occasional omissions. Lungs unchanged, except for the addition of a few piping râles over the affected areas. Patellar reflex +, ankle clonus distinct. Decubitus over sacrum.

August 25th. General condition better. Still delirious. Slept more. Breath sounds over entire right chest short and weak.

August 26th. Condition better. Pulse regular. Liver palpable a finger's breadth below costal margin.

August 27th. Slept poorly, but there is less delirium. Lung findings the same. Patellar reflex and clonus unchanged.

August 28th. Liver margin no longer palpable.

August 29th and 30th. Condition unchanged.

August 31st. Bleeding from gums during night. Slept poorly. Sensations of oppression over chest. Feels better and is quieter.

September 1st. General condition better and slept more soundly. Appetite increasing. Gums reddened and covered with blood in places. Regional nodes no longer palpable, axillary groups smaller, but still slightly tender. Breath sounds on the right are short, rough, but loud. Liver and spleen not felt. Miliaria over trunk and abdomen, together with many petechiae. Muscle tremor distinctly elicited by tapping. Patellar reflex present, no ankle clonus. Blood culture is negative.

September 2nd. General condition better. Petechiae have disappeared. Gingival bleeding has ceased.

September 3rd. Unchanged.

September 4th. Profuse sweating after sleep.

September 5th. Unchanged.

September 6th. Unchanged. Body weight now 41 kgm., has lost 13.6 kgm.

September 7th. Unchanged.

September 8th. Condition better, pulse slightly accelerated, patellar and Achilles reflexes are increased.

September 9th. Appetite better, pulse rapid, with an occasional dropped beat, but strong. Palate coated with patches which are easily removed. Abdomen distended, tympanitic and tender, but no resistance.

September 10th. Unchanged.

September 11th. Patches on palate have gone, general condition better.

September 12th. Pulse rapid and regular.

September 13th. Unchanged.

September 14th. Discharged from hospital.

iv. Severe Case (Died). Male, 30, farmer. (Fig. 6.)

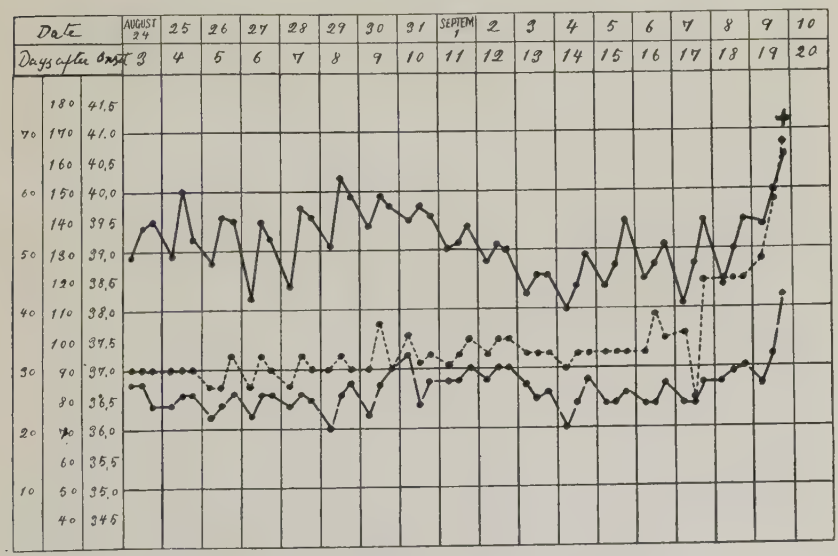


Figure 6

Severe Case. (Died). Male, 30 years old. Farmer

History: The patient had never been bitten by akamushi, nor had tsutsugamushi disease. On August 14th, and 20th, 1915, he went into the endemic region and a few days later noticed tenderness and swelling of the right femoral nodes. As he thought he had been bitten on the outer side of the right tibia by a mosquito he attributed these symptoms to the insect bite. Being warned by a friend, on the 23rd, that this might be an akamushi and not a mosquito bite, he entered the hospital on the 24th. The onset of his illness was at noon on the 22nd, with headache and tired-feeling. The same evening he had a chill. He was constipated from the outset.

Physical Examination: The face is flushed, the lids and conjunctivae are congested. Pharyngeal mucosa and gums reddened. Cracks at both angles of the mouth. Pulse large, regular, 92. Relative dullness of the liver at the fifth rib, absolute at the sixth. Right margin of heart at the right border of the sternum, its base at the third rib, the apical beat seen in the fourth interspace, just within the nipple line. Heart sounds pure. Lungs normal. Spleen not felt. No abdominal tenderness. Patellar reflex weak, Achilles reflex distinctly noted. There are doubtful papular eruptions on the sides of the chest, and a red sudaminal eruption on the neck, shoulders, arms and thighs. The eruptions on the flexor surfaces of the arms and extensor surfaces of the thighs are pustular. During the examination "goose-flesh" appears and the patient shivers.

On the right leg, about 15 cm. above the sole, there is an elevated reddish area, about 1.3 cm. in size, neither indurated nor tender, and at its center there is a dark-red crust, 2 x 4 mm. in extent. The left submaxillary nodes are about 3 mm. in diameter, those of the right side about 7 mm. The right cervical nodes are 5 mm. in size and not very hard, those on the left are not palpable. Many of the right axillary group are swollen and hard, the left are about 1.5 cm. in diameter and tense, but not hard. The right inguinal nodes are swollen, but not tender. One of the femoral group measures about 5 x 4 cm., another 2.5 x 1.5 cm., and a third 1.0 x 0.5 cm.; all of them are tender and hard. The left inguinal nodes measure 0.6 cm. in diameter and are hard, but not tender. The left femoral are about 1.2 cm. in diameter, also hard but not tender. The popliteal and epitrochlear nodes are not swollen.

Course of the Illness: August 25th. There is insomnia, he complains of headache. His eyes are bright, his tongue coated, no tremor. Pharyngeal and palatal mucosa are congested. Reddish papular eruption over abdomen, forearm and flexor surface of the thighs, but none on the face. Cervical nodes are 0.6 cm. in diameter and tender. The right femoral nodes are very tender. At 7 P. M. one of these was excised under novocain.

August 26th. Facial expression normal, profuse sweating. No conjunctival congestion. Eyes bright. No congestion of the pharyngeal mucosa. Two papules present on the palate, one with a yellowish spot at its center. Pulse large and regular. Patellar and Achilles reflexes increased. Some tenderness of the right submaxillary nodes. The right cervical now averages 1.2 cm. and are hard; the left 1 x 0.7 cm., soft but not very tender. The right axillary nodes average 0.6 cm. and are hard and slightly tender, the left are 1.7 cm. in size, are soft and flat, some of them being tender. The epitrochlears are not swollen. The left femoral and inguinal nodes are as they were yesterday. The right not examined because of the dressing. Popliteal nodes are normal.

The eruption is distributed as follows: Head—there are a few red papules on the forehead and chin, some scattered on the cheeks and eyelids, none about the month. Trunk—a typical eruption over the chest, fewer papules over the sides, many on the shoulders, and a few over the back and on the waist-line; occasional papules on the buttocks, and many over the abdomen. Arms—Right—A few papules on

the right arm, practically none at the elbow, many on the forearm, a few on the dorsum of the hand, none on the palm. Left—A few papules on the extensor surface of the arm and at the bend of the elbow, many on both surfaces of the forearm, none on the hand. Lower limbs—Many papules on both thighs and about the left knee. Fairly numerous on the left leg. There is a dark-colored crust at the site of the insect-bite, which is larger than it was and is surrounded by desquamation, but not indurated.

August 27th. Insomnia. Facial expression normal, conjunctivae congested, tongue moist and coated grayish-white. Pharyngeal mucosa much congested. Palate congested and shows an eruption. Both submaxillary, the left inguinal, and the femoral nodes are now somewhat tender. The eruption on the face has increased and is typical. It has increased elsewhere, but that on the left forearm has begun to be discolored.

August 28th. The eruption on the palate now measures 1.5 cm. in diameter. The gums are soft and red. The pulse is large, dicrotic, regular. Patellar reflex increased. No ankle clonus. Lymphnodes all tender. The eruption has spread to the scalp, there are many papules on the cheeks and chin, occasional lesions in the submental region and around the mouth. The distribution has not changed much, except that the palms have become involved and there are more papules on the legs, the popliteal region and calf now showing a few. Some have appeared on the feet, but the soles are not distinctly involved.

August 29th. Somewhat expressionless, flushed, marked congestion of conjunctivae. Tongue grayish-white and tremulous. Deafness and tinnitus are present. Pulse large, dicrotic, but regular. Both submaxillary groups of lymphnodes are tender. Eruption unchanged. Many sudamina on chest and neck.

August 30th. There is an anxious expression, the conjunctivae and nasal mucosa are congested. The tongue is still coated. Palatal mucosa congested, enanthem still present. The eruption on the head is becoming discolored, some of the large papules on the face are 9 mm. in diameter, partly brown, with light brown crusts. Some appear flat and shiny. Large papules on the forearms measure 1.0 to 1.2 cm. Some of the eruptive lesions over the scapular region are 8 mm. to 1.2 cm. in size. Those on the waist and back are brownish. The largest on the thigh is 1.2 cm. in diameter.

August 31st. Expressionless. Pulse moderately strong. Conjunctivae and nasal mucosa intensely congested. Palate diffusely reddened by the enanthem, at the center of its papules are small vesicles. Pharyngeal mucosa congested, red. Heart and lungs normal. Eruption is becoming distinctly discolored.

September 1st. Face, conjunctivae, tongue and pharynx appear as before. Heart and lungs normal. Spleen not felt. Pain near umbilicus after eating, deep palpation dislodges gas bubbles. Patellar and Achilles reflexes weak. Submaxillary nodes tender. Cervical, axillary and femoral nodes slightly so. The entire eruption has almost faded away. At 3 P. M. 3 c. c. of serum taken from a convalescent patient was administered intravenously.

September 2nd. General condition slightly better. Expressionless. Face, tongue, palate, and pharynx appear as before. Lips are dry. Pulse large, and regular, not dicrotic. Heart and lungs normal. Spleen not palpable. Right patellar reflex absent, left weak, both Achilles reflexes weak. Both submaxillary and axillary nodes are tender, but the rest are not. Most of the eruption had faded out and there remain light brown slightly elevated spots, surmounted by crusts and areas of desquamation. At 5 P. M. 3 c. c. of convalescent serum was again given intravenously.

September 3rd. General condition good. Expressionless. Marked

conjunctival congestion. Lips dry, tongue coated. There are small ulcers at the edges of the areas of congestion on the palate. Pharynx greatly congested, gums red and rough. Pulse large and regular. Heart and lungs normal. Spleen now palpable. Both patellar reflexes weak. Achilles reflex weak. Submaxillary nodes, alone, are tender. The site of the insect bite is round, dark-brown, with sharp, reddened edges and about 6 mm. in diameter. No induration. At 4 P. M. three c. c. of convalescent serum injected intravenously.

September 4th. General condition better. Face edematous and flushed. Conjunctivae congested. Tongue moist, coated, yellowish-gray. Pharynx congested. Heart and lungs unchanged. Spleen hard and palpable. Right submaxillary nodes still tender. The application of a rubber tourniquet to the right arm for ten minutes causes petechial hemorrhages into the spots representing the eruption.

September 5th. In a somnolent condition, face flushed and edematous. Conjunctivae, tongue, palate, and pharynx unchanged.

September 6th. During the forenoon the patient felt better, but lapsed into somnolence this afternoon. Lips dry and cracked. Pulse regular, with moderate expansion. Right tibia tender. Almost no patellar reflex. Weak Achilles reflex.

September 7th. Slept better and is feeling a little better. Facial expression tranquil. No patellar or Achilles reflexes elicited. Hiccough started this morning, becoming severer towards evening. Somnolent.

September 8th. Drowsy, talking deliriously, but answers questions. Pulse fair, face edematous and fingers tremulous. Tendon-jerks present. Lips dry, tongue coated. Palate congested and small ulcerations are seen. Respiration is stertorous. Heart and lungs normal. The spleen is palpable and hard. Feet edematous. The wound at the site of the insect-bite is dry at its base.

September 9th. General condition poor. Semiconscious, restless, with talkative delirium. Pulse very rapid, expansion poor. Fingers tremulous and tension-jerks present. Breath-sounds short and rough at the bases behind and fine bronchial râles are heard. Spleen not felt.

At 8 P. M. the patient's breathing became shallow and rapid and he was mentally confused. Digalen 0.7 c. c.; camphorated oil, 9.0 c. c., and salt solution, 600 c. c. injected without results. The patient died at 9:30 P. M.

v. Very Severe Case, (Died). Female, 32, farmwork. (Fig. 7.)

History: The patient had never had tsutsugamushi disease or been bitten by akamushi. On July 15th and 17th, 1917, she worked in an endemic region, becoming very ill on the evening of the 20th. The next day constipation appeared and has continued. She entered the hospital on the afternoon of the 22nd.

Physical Examination: Well-developed and well-nourished woman who is apparently suffering intensely. She is flushed, her eyes are bright, her breathing is rapid. Pulse regular, weak, 138; temperature 40.3° C. Conjunctivae much reddened. Nasal mucosa is congested. The lips are dry, the tongue moist and coated, the pharynx congested.

Absolute liver dullness at sixth rib. Upper border of heart is the third space and its apex in the fourth, one finger's breadth within the nipple line. The right border is at the midline of the sternum. Heart and lungs normal. Spleen not palpable. Patellar reflex increased. Skin dry and hot. A few areas of eruption are seen on the chest and upper arms. On the right scapular region there is an elevated red spot measuring 0.9 x 1.2 cm. Its margin is vague and its center is covered by a yellowish crust. The right cervical nodes are the size of peas and soft; the supraclavicular the same size, hard, and slightly tender. The right axillary nodes average 1.5 cm. in size, those on the left are

about 1.0 cm. in diameter and not tender. Epitrochlears not felt. The right inguinal nodes measure about 0.7 cm. and are hard; the left ones are small. The right femoral nodes are about 0.7 cm. in diameter and are hard, the left are about 1.0 cm. in average diameter and soft.

Course of the Illness: July 23rd. Profuse sweating, pulse large and expansile.

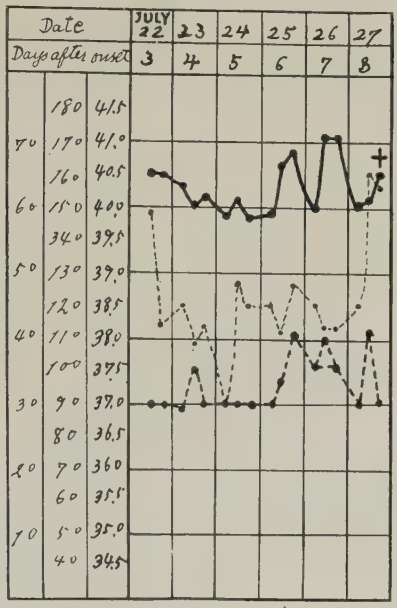


Figure 7
Very Severe Case. (Died). Female, age 32. Farmer

July 24th. Sleeps poorly and is excited, pulse moderately rapid.

July 25th. Pulse moderately rapid and of poorer quality. Many râles are heard at both bases in front. Spleen not felt. By evening, the general condition has become poor.

July 26th. Conscious during morning, but in poor condition. Pulse rapid and regular. Tongue moist and coated. The regional nodes are no longer tender. Spleen now palpable. During the evening the patient became noisy and excited, attempting to stand up in bed. A copper-colored eruption has appeared on the arms. The pharynx is slightly congested. Piping rhonchi and râles are heard on both sides of the chest. Patellar reflex +.

July 27th. During the morning the pulse is rapid and weak. Mental condition unchanged. Eruption fading, tongue dry and coated. Patellar reflex +. During the evening the general condition is poor, pulse small and weak. Camphorated oil and morphin administered. Breathing shallow and rapid. Died 3 A. M. on the 28th.

A blood examination on the 24th showed the following:

Leucocytes: 6,450.

Differential: Neutrophiles—Unsegmented nuclei, 58.8%, lobulated nuclei 27.0%; immature, 0.2%; metamyelocytes, 0%; total, 86%. Lymphocytes—Large, 2.4%, Small, 10.5%; total, 12.9%. Large mononuclears—2.2%, Transitionals—0.7%. Eosinophils—0.4%. Basophils—0%. Türk cells — 0.4%, Nuclear shadows — six. No poikilocytosis, anisocytosis, or polychromatophilis.

B. General Discussion of Each Symptom.

a. Fever.

The typical fever of tsutsugamushi disease may be divided into four stages: a, the invasive; b, the fastigial; c, that of defervescence, and d, that of convalescence. Antipyretics were used in no case, cardiac stimulants being employed instead. In mild cases with atypical fever-curves, there are not four distinct stages, and in many of the cases of second attack the fever does not run a typical course. It may be remittent from the beginning, varying between 37° and 39° C., sometimes exceeding 39° C. It may last several (up to ten) days and then take on the convalescent type. In all cases there is a lesion at the site of the akamushi-bite and tenderness and swelling of the regional nodes. Eruption is seen in many cases, but may be absent.

In moderate or severe cases one may distinguish the four stages of fever. The bite lesion, tender nodes, and an eruption are always present.

The stage of invasion lasts from 3 to 5, occasionally 7 or 8 days, when the fastigium begins. As in typhoid fever, the temperature may fall to 38° or 39° in the morning, or as low as 37° during the first few days. In the evening it may rise to 39° or 40°, or even to 40.5°. It then becomes continuous for from five to ten days. Sometimes it may last only three or four. In fatal cases the fever often reached 41.8°. It may be remittent, instead of continuous and when the remittence is frequent, it is difficult to distinguish the fastigium from the invasive stage.

During defervescence the fever falls by lysis to 37° C. in the course of five to seven days. In mild cases lysis may require but three or four days, while in severe cases it may take ten days to two weeks. During this stage, on account of complications which often intrude—such as pneumonia, gangrenous stomatitis, suppurative lymphadenitis, and cystitis —the typical curve may be changed and may again rise, as the course of the fever is prolonged.

Only a few cases were observed during convalescence, four of these were in our college hospital for a long time. In mild, or moderately severe cases without complications,

the normal temperature is maintained during convalescence, or it may stay around 37.4° to 37.5° for a few days before normal temperature is restored. On the other hand, in severe cases, the fever may run an intermittent course for fifteen or more days. One of our three severe cases had an intermittent fever of 36.4° to 39.3° for nineteen days, on the eighteenth a temperature of 40° C. In the second case there was a temperature of 36.2° to 38.5° for eighteen days; in the third 36.4° to 38.2° for eighteen days. In all cases the fever fell by lysis. Occasionally there were cases in which the temperature fell below normal during the convalescent stage; in one case (male, 48 years) to 35.3°, but in two or three days it became normal. A fourth case (male, 34 years) complicated by pneumonia, showed an intermittent fever, the temperature falling several times to 35.5° C.

b. Circulatory System.

i. Pulse: The pulse rate is low when compared with the temperature. In the stage of invasion, with a temperature of 40° C. the pulse rate in strong men will be 70-100, in old people, women, or children it may be 120-130. During the fastigium, with the temperature at 38°-40.6°, the rate will be 75-100, occasionally 100-125 in men. As a rule it averages ten to twenty beats higher in old people, women, and children. During defervescence the rate is lower in comparison to the fever, but five to ten beats faster than during the fastigium. When the rate increases to 140, or more, the prognosis is bad, and in this case pulse rate is proportionate to fever.

During the stage of convalescence the pulse rate corresponds to the temperature, but occasionally may be slow. Five out of fifty-seven cases who had the disease for the first time, had a rate of 55, or even 35, which lasted usually for two or three days. In one case, complicated by pneumonia, there was a pulse rate of 40-50, on one occasion 35. This bradycardia lasted for eight days. It does not necessarily correspond to the severity of the attack, being seen in moderate cases and even during second attacks.

The character of the pulse is always regular, large, and often dicrotic. Dicrotism may occur during the stage of

invasion, but is usually noted during the fastigium, continuing into the stage of defervescence. When the heart weakens the pulse becomes irregular and its expansion poor. A rapid pulse is occasionally encountered.

ii. Heart Function: As a rule a systolic murmur is heard at the apex or over the entire praecordium, and the second pulmonic sound is accentuated and the heart activity increased. When the heart weakens its area of dullness extends to the left, or simultaneously to the right. In such cases oedema may appear in the feet, the eyelids also becoming oedematous in severe cases. There was oedema of the feet and legs in 21 out of 57 cases of first attack, of the face (especially the eyelids) in 10. In a few cases there may be oedema of the face without the feet or legs being affected. It sets in during the latter half of the fastigium, or during defervescence.

iii. Blood Pressure: The blood pressure was taken in ten cases with the Riva-Rocci apparatus.

Day	1 No. 28	2 No. 26	3 No. 21	4 No. 5	5 No. 43	6 No. 28	7 No. 13	8 No. 57	9 No. 32	10 No. 23
1	..	..	..	..	110	..	..	..	..	..
2	..	..	..	..	110	..	..	..	..	..
3	..	..	..	..	102	..	..	..	..	108
4	..	..	..	..	95	..	..	106	98	100
5	..	119	..	..	..	..	92	100	97	105
6	..	..	..	..	..	..	87	94	95	..
7	..	..	..	..	92	..	85	96	90	..
8	..	95	..	..	85	..	85	87	86	96
9	..	93	..	..	86	..	73	88	86	94
10	..	86	..	..	84	82	77	..	88	85
11	..	90	..	..	80	82	68	..	77	90
12	..	85	..	87	81	76	75	78	82	90
13	..	85	..	82	..	72	72	88	80	76
14	..	83	87	77	..	65	69	..	76	81
15	..	..	77	72	83	81	76	..	86	76
16	85	75	80	73	84	85	80	..	81	75
17	82	74	83	75	..	86	82	..	81	74
18	83	..	82	73	..	91	78	..	..	..
19	75	..	81	73	..	100	80	..	..	..
20	70	..	80	72	..	95	85	..	..	..
21	76	..	85	84	..	91	..	..	..	..
22	73	..	78	..	..	94	..	..	..	..
23	81	..	90	..	..	..	..	..	..	..
24	76	..	95	..	..	..	..	..	..	..
25	83	..	..	..	..	82	..	..	..	..
26	..	..	..	..	..	..	..	..	..	..
27	..	..	..	..	..	94	..	..	..	.
28	..	..	..	..	..	101	..	..	..	.

As the table shows, the blood pressure lies between 100 and 119 for the first few days after the onset, but it gradually falls below 100 and, at the end of the fastigium or the beginning of defervescence, lies between 90 and 100, becoming normal during convalescence.

iv. Blood Viscosity: This was determined in seven cases with the Hess viscosimeter. Viscosity is lowered in the early stage of the disease; in men it is between 3.7 and 3.9, in women between 3.0 and 3.7. It becomes normal during defervescence or convalescence. Healthy normal men show a reading of 5.2, women of 4.2 to 4.5, so that the figures just given show a notably decreased viscosity during the fastigium.

v. Blood Coagulability: This is often decreased during the fastigium and we have often had difficulty in controlling hemorrhage while removing lymphnodes for examination. In one girl of seven a left axillary node was excised on the day of onset and fifteen days later, when the wound had healed completely, a swelling about the size of a walnut was noticed. On incision it proved to be an hematoma; following the incision open bleeding began and, in spite of recourse to every known remedial measure, the child died from hemorrhage. (Fig. 8.)

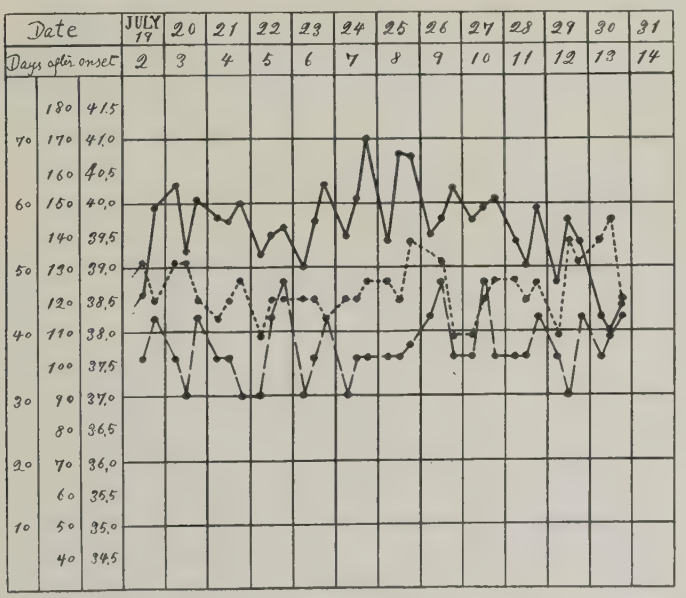


Figure 8

vi. Other Blood Findings: No systematic studies of the blood have ever been made in the case of tsutsugamushi disease. Tanaka reported decreased coagulability and stated that there is no morphological change in the red cells. The number of erythrocytes varies from 4,800,000 to 5,200,000; that of the leukocytes from 6,000 to 8,000. Hemoglobin is also decreased. It reads 40-70% with the Fleischl hemoglobinometer. Hayashi examined five typical cases between six and nineteen days after the onset and reported the number of red and white corpuscles to be decreased to one-third, or one-half the normal count. (Red cells 2,360,000 to 4,101,000, white cells 2,734 to 5,311.) These investigators have disregarded the differential count. Sawada (157), however, reported that in four cases there was a decrease of polymorphonuclear neutrophils and transitionals, leaving the leukocytes mostly lymphocytes and large mononuclears. He found no other pathological changes in the leukocytes. The number of red cells in one case was 3,700,000 and of white cells in another case 4,062. These incomplete examinations, then, show that there is leukopenia in this disease. While Hayashi and Sawada find a decrease in the number of red cells, Tanaka finds no change. There are no other detailed studies of the blood in this condition.

As a part of our clinical study I have therefore systematically examined the blood of many patients; not only this, but I have also examined that of Japanese monkeys *(Macacus fuscatus)* as controls. These had had injections of blood from patients for experimental purposes and I followed the changes in their blood throughout the course of the disease.

It is well-known that intestinal parasites influence the blood-picture; we therefore carefully examined human and monkey feces several times by the antiformin-ether and occasionally by the bone charcoal incubation method.

Methods of Examination: Blood specimens were taken from fasting patients or monkeys, from the ear and the following examinations made: 1. Fresh blood, 2. Cell counts, 3. Fixed film, 4. Hemolglobin determination.

Fresh blood was examined under the dark field either at room temperature or in Nicoll's constant temperature cham-

ber. The blood was either undiluted, or diluted with saline solution, or sodium citrate.

Cell counts were made in the Zeiss counting chamber and the hemoglobin was determined with the Sahli aparatus.

Fixed films were made in the usual way by fixing either in absolute alcohol, methyl alcohol, formalin, or by heating. The following stains were used: Romanowsky, Giemsa, Leishman, May-Grünwald, the triacid, and methylen-blue.

In all, sixty cases coming from three endemic localities were examined; of forty-two first attacks, where the illness ran a typical course, twenty-eight cases were examined a dozen times, or more.

Early invasion to convalescence, 9 cases.

Latter part of invasion to convalescence, 14 cases.

Defervescence to convalescence, 5 cases.

Two or three examinations were made in each of the rest between the invasion and defervescence, between the fastigium and defervescence, and between invasion and fastigium. One or two examinations were made in a case during early invasion, in two cases in the latter half of invasion, seven cases during the fastigium, and one during defervescence; fourteen cases being tested at varying periods. One or more examinations were performed in four mild, and seven severe cases. As controls, the blood of several individuals bitten by akamushi, but not infected with tsutsugamushi disease, was examined.

Discussion of Observations on Blood.

(1) Erythrocytes: Poikilocytosis, normoblasts, and polychromatophilia were observed in a few instances; they are not characteristic of the disease but due, rather, to the anemia that accompanies it. There are no other marked changes.

In twenty-two out of the thirty-one cases examined there was more or less decrease in the number of erythrocytes. It begins to fall in the latter half of the fastigium and during defervescence, gradually rising again during convalescence. It may fall from 4,500,000 or 5,200,000 to 3,400,000 or 4,000,000 recovering to normal in two weeks; or it may take four or five to do so.

(2) Hemoglobin: As a rule this is decreased during the course of the illness. The maximum fall is seen in many cases between the middle of the fastigium and defervescence. In one case the index fell to 0.72, but in the majority of cases it was 1.0 to 0.85. Sometimes it rose to normal late in the period of defervescence, but it usually rises in that of convalescence. There were a few cases in which a low index persisted for two or three weeks. This decrease in hemoglobin parallels that in the number of red cells, but there were a few cases in which the hemoglobin index was very low.

(3) Leukocytes: Leucopenia is characteristic of this disease. There were three cases in which our observations began early in the onset: two female, 7 and 46 yrs. respectively, and one male, 57 yrs. There was no leucopenia in the first two, in the third a slight leucopenia was already present. On the fifth day the girl's white count was 2,100, that of the man was 2,486 on the sixth day. Three cases in which observation was begun on the second day (males, aged 19, 43, and 23) showed the lowest count as follows: the first had 4,500; the second 3,180 on the thirteenth day; the third, 1,490 on the fourteenth day. In eight cases observation was begun on the third day; males - 13, 20, 23, 24. 41 and 67 yrs. old; females - 13 and 25. The lowest counts of the 13, 20 and 23-year old males were 2,730 and 4,500, and 3,670 respectively. In three of the other five, blood-counts were made throughout the illness. The lowest of the 25-year-old female was obtained on the sixth day, 2,640; that of the 13-year-old girl on the sixth day was 4,850.

Examination of thiry cases throughout the entire course of the illness showed the lowest white-counts between the sixth and ninth days after onset in eighteen cases, the third and fifth in six, and the second and twelfth respectively in two. As a rule the minimum was rapidly reached between the sixth and ninth days after onset. This period corresponds to the fastigium, the first half of the period of continuous fever, and that in which there is the most extensive eruption. In a few instances the maximum leucopenia precedes the eruption, occuring during the invasion; or it may appear after the eruption has faded, in the latter half of the

fastigium. This leukopenia usually lies between 3,000 and 5,000; less often between 2,000 and 3,000; and only a few cases show the extremes of 1,000 - 2,000 and 5,000 - 6,000. The lowest counts obtained were 1,488 in a man of twenty-three and 2,100 in a girl of seven.

The white-count usually resumes a normal figure during the latter half of defervescence or during convalescence. At this time there is often a leukocytosis. As our patients were discharged from the hospital in this very period, we examined only eighteen out of the thirty patients under discussion. In ten leukocytosis appeared in the defervescent stage, twelve to nineteen days after onset. One exception was a man of 34 in whom leukocytosis appeared on the ninth day after onset and reached a maximum on the eleventh. This case had his lowest white-count on the third day. In the other six cases the leukocytosis occurred between the second to fourteenth days of convalescence. There were eleven cases in which the leukocytes exceeded 10,000, in the other seven it lay between 8,480 and 9,080. We considered anything over 8,000 as a leukocytosis, anything under 6,000 as a leukopenia.

Of the eleven cases with white-counts exceeding 10,000, five lay near 10,000 and six over 13,000, the highest count being 13,900. There were, however, three cases in which there was no leukocytosis in the convalescent stage; two complicated by pregnancy and one by pneumonia.

The degree of leukopenia does not necessarily correspond to the severity of the illness; in four fatal cases there were counts of 2,100 to 3,750 three to six days after onset, while in five they were between 5,850 and 6,450 between the fifth and tenth days. Moreover, in the case of a 30-year-old male, although observation was begun on the fifth day, there was no leukopenia at all. Between the fifth and thirteenth days after onset the count was 6,170 to 7,420; but blood-counts made later in the disease showed a distinct, post-infectious leukocytosis. In a few cases complicated by catarrhal pneumonia, or by suppurative lymphadenitis, the counts were not much altered; seemingly there was no reactive leukocytosis.

There was no leukopenia in the case of the few people we examined who were free from tsutsugamushi disease but had been bitten by akamushi.

We may state, therefore, that there is a leukopenia in tsutsugamushi disease. The white-count decreases from the invasion to the fastigium, reaching its minimum at the period of eruption. There is a slight leukocytosis in the majority of cases in the latter part of the fastigium and in the period of convalescence, the count then gradually returning to normal. The degree of leukopenia does not necessarily correspond to the severity of the disease, it may depend upon individual differences, or upon the condition of the blood-forming organs. Changes in the bone-marrow and lymphoid tissue are more pronounced in this disease than in typhoid. There is usually no leukocytosis in response to complications such as catarrhal pneumonia or pyogenic lymphadenitis.

(4) Changes in the leukocytes: (Description based upon the sytematic study of twenty-eight cases.)

Polymorphonuclear neutrophils: The relative number of these is increased from the earliest stage of the disease onward, being most marked during the fastigium. The maximum is reached soon after the total count has reached its minimum. In many cases it is 80%, often it is 90%; in one it was 93.8%. The highest percentages are found in the latter half of the fastigium, or early in defervescence.

During the early stages of the disease the decrease in lymphocytes is not as marked as that in polymorphonuclear neutrophils, we may therefore assume that the latter suffer the most injury. There are very few cases in which the absolute number of polymorphonuclears reaches 3,900; usually there are from 2,000 to 3,000; sometimes only 1,260 to 1,670. Thus there is an absolute polymorphonuclear leukopenia and this relation is maintained until the total white-count reaches its minimum. Then, with the gradual decrease in the number of lymphocytes, the absolute number of polymorphonuclears increases until it exceeds the normal count. Next, on account of a rapid increase in the number of lymphocytes, the relative percentage of polymorphonuclears falls below 60%, to 40 - 50%. In a severe case it fell to 37.1%, about half the normal. During defervescence the absolute figure exceeds the normal in patients who have leukocytosis, otherwise it is below normal. During convalescence, when there is usually a leukocytosis, the absolute

number of polymorphonuclears is also increased. With a return to the normal white count the percentage of lymphocytes likewise becomes normal. During the fastigium, even without marked leukopenia, the relative number of polymorphonuclears is increased; while that of the lymphocytes is decreased.

According to Arneth, who studied the alterations in the nucleus of neutrophil cells, the granular neutrophils are derived from the granular neutrophil myelocytes and the nucleus is at first bean, or kidney shaped, gradually becoming indented until lobules are reduced and polymorphonuclear cells are formed. He considers that the greater are the number of nuclear lobes, the older is the cell, and classifies the neutrophils according to the shape and number of nuclear lobes. In infectious diseases, there is an increase in the relative number of so-called "Arneth immature polymorphonuclear neutrophils," those in which there are few nuclear lobes, and this phenomenon has been called "the shifting of neutrophil blood picture to the left." It is explained by the theory that, during infection, a large number of polymorphonuclears is destroyed in the circulation and that comparatively immature cells make their appearance as a consequence. As this classification is very complicated, Pappenheim recommends recognizing granular neutrophil myelocytes, metamyelocytes and polymorphonuclear neutrophils.

In 1911, Schilling and Torgau (162) noted the appearance of rod-shaped neutrophil granules and atrophic, ill-defined nuclei in the polymorphonuclears of normal blood during certain infectious diseases and, considering them to be regenerated young cells, opposed Arneth's views. As these cells appear in the circulation as a result of toxic disturbances of bone-marrow activity they have been called "degenerative rod-nucleus leukocytes," and their appearance in large numbers a "degenerative nuclear shifting."

When the regenerative process becomes vigorous, after a toxin has disappeared, a small number of myelocytes and so-called "metamyelocytes," may appear in the blood and this is termed "regenerative nuclear shifting." If the foregoing nomenclature be adopted, the classification according to nu-

clear changes becomes simple and more accurate, and we have adopted it in several cases. (See tables)

Neutrophil myelocytes appear in all our cases, excepting a few, during the latter half of the fastigium, or in the defervescent or convalescent stages, up to a short time before the blood-picture becomes normal. The number of these cells is usually 0.3 - 0.7% of the total, occasionally as high as 3%. There was one case in which it touched 4.2%. They appear after the polymorphonuclear-count has reached its maximum, attain their own at the beginning of the convalescent period, and then gradually decrease in number.

Metamyelocytes: The appearance of these immature neutrophil granular cells precedes that of the neutrophil granular myelocyte in the fastigium. They appear almost invariably, reaching a maximum in the latter half of defervescence or the beginning of convalescence, and then decrease gradually. Their percentage averages 5%, but there was one case in which it reached 8.7%. In a few fatal cases, in which observations were made only during the fastigium, the appearance of metamyelocytes preceded that of the myelocytes.

Degenerative leukocytes with unsegmented nuclei appear while the eruption is at its maximum and increase in number until it fades. The usual percentage of these cells is 20 - 50%, often higher. In a normal, healthy man it is usually 4 - 5%. In one marked instance it reached 63.8%, the polymorphonuclear leucocytes falling to only 10.6%. The percentage of degenerative forms gradually decreases during defervescence. Thus, the blood of a tsutsugamushi disease patient shows a marked degree of nuclear shifting. In many cases myelocytes and metamyelocytes appear while the eruption is fading, indicating a slight regenerative nuclear shift. In mild cases this may be due to the stimulation of the regenerative functons of the marrow cells, but in a severe case the marrow-function is so disturbed that immature cells are constantly thrown into the circulation. There are marked changes in the marrow of tsutsugamushi disease patients, as will be shown later on.

Lymphocytes: Numerical change in the lymphocytes is a characteristic of this disease; their decrease in the early stage is not very marked, but during the fastigium it becomes

so. This decrease continues throughout the period of marked leukopenia and reaches a minimum in the latter half of the fastigium, or early in the defervescent period. A rapid increase then follows and, by the latter half of defervescence, or during convalescence, there is lymphocytosis. From this peak the count slowly falls to normal. At its lowest, the percentage of lymphocytes is often about 10% and there are cases where it reaches 4.3 - 5.5%. The absolute number of lymphocytes is usually less than 900, in many instances it may be about 500. There was one case in which it reached 160. (Normal: 1,380) Thus, both the absolute and relative numbers indicate lymphopenia. If there be leukocytosis, however, both the absolute and relative numbers indicate lymphocytosis. At the highest, the percentage is in many cases 30 - 40% and there were a few in which it reached 50.7%, or even 62%. In a few instances, in which recovery was exceptionally slow, the lymphocytosis was not marked, the count being within normal limits. The absolute number, in many cases, ranged from 2,000 - 3,000, or often higher, in one instance, 130.

It is interesting that the blood examination of several very severe cases dying during the fastigium showed marked lymphopenia during that stage, when leukopenia was absent, and polymorphonuclear leukocytes were increased.

Eosinophils: The decrease in eosinophils in the early stage is very marked; by the first to third day after onset they have fallen to 0.2 - 1.0%, or entirely disappeared. They always disappear during the fastigium. This picture continues to the stage when the eruption fades, or to that of defervescence, when their percentage gradually increases and returns to normal during convalescence. There is, however, no post-infectious eosinophilia as in typhoid.

Basophils: In the early stage of eruption as many as 0.3 - 0.5% may be found, but in the fastigium they disappear completely as do the eosinophils. When these reappear, those also increase in number and, during the convalescent stage they may form 0.5 - 0.9%, occasionally 1.2% of the total.

Transitionals and Large Mononuclears: The changes in these varieties of leukocyte are not marked; as a rule, they decrease more or less during the fastigium, their percentage

lying between 2 and 3%, but during defervescence or convalescence they resume their normal percentage of 5 - 9%. In the case of a thirty-year old woman, however, there was a marked increase in these cells during the first two weeks of the convalescent period, to 28.6%, which is exceptionally high.

Türk Cells: This type appears at the time when the eruption fades, occasionally it may be noted during very early stages. It is always seen at some time during the course of the illness, usually the percentage is 2 - 3%, occasionally 5 - 7%. It decreases comparatively rapidly during convalescence.

vii. Blood Findings in Atypical Cases and Cases of the Second Attack.

The blood pictures in cases of second attack are about the same as those seen in first attacks, but the illness is milder and the changes in the blood-picture correspondingly mild. The number of leukocytes is usually above 4,000, though it may fall to 2,500. The increase in neutrophils is not marked and the decrease in lymphocytes is slight, in fact there is usually lymphocytosis. There was one case in which the percentage of lymphocytes exceeded 50%. Basophils almost disappear in all cases, but eosinophils are found more or less generally, although their number is markedly decreased. There are no changes in the transitional or large mononuclear leukocytes, but a few myelocytes, metamyelocytes and Türk cells may appear in the blood.

In atypical cases the blood picture is not constant; there may be either increase or decrease in the leukocytes, immature marrow cells do not appear, and basophils are absent.

c. Lesion at the Site of the Akamushi-bite.

The focal skin necrosis, supposed to be the site of invasion by the virus of tsutsugamushi disease, is not caused by this virus at all, but by the secretions of the akamushi. This necrotic focus is one of the important signs in diagnosing the disease. If one see this lesion and simultaneously find tenderness of the regional lymphatics even the layman finds no difficulty in making a diagnosis. Not a single case out of ninety patients under my observation failed to have these two signs. Two out of twelve cases of second attack showed atypical

bite lesions. In the majority of cases these are single, but there may be two or three; out of ninety cases in my series, eighty had one, eight had two and two had three lesions.

Region	Total	Right	Left
Scrotum	12		
Back	7		
Thigh	7	3	4
Abdomen	6		
Buttock	5		
Legs	4	2	2
Chest	3		
Ear	2		
Neck	1		
Forearm	1	1	
Axilla	11	7	4
Waist	7		
Groin	6	3	3
Nape	6		
Arm	4	1	3
Perineum	3		
Penis	2		
Canthus eye	2		
Knee	1	1	
Nasolabial fold	1		

The bite lesion may be found anywhere on the body, but the largest percentage is found on the genitalia and neighboring parts, and in the axilla. Next in order of frequency come: back, abdomen, waist, and buttocks.

That there is a close relationshp between the bite of akamushi and localized skin necrosis has been claimed by other investigators (Tanaka, Miyajima, Nagayo, (115) and Hayashi). Bälz, in 1878, said that this is not caused by the bite of the akamushi, but by the invasion of a miasma; this, like the miasma-theory of malaria, is an exploded hypothesis. We have six cases in which the history clearly indicates that the wound was caused by the bite of the akamushi; in these cases the mites were either removed, or left in situ and a prickling sensation came on at the site of the bite. Later, necrosis of the skin ensued.

That the bite of akamushi causes tsutsugamushi disease is conclusively proved by the following case. A woman of twenty-eight was bitten by an akamushi in the right axilla, two hours later the entire region of the bite was excised by a physician. The incision took on the appearance of the usual bite lesion, there was regional lymphadenitis on the ninth day, and on the eleventh symptoms of the disease appeared

and the illness ran a typical and moderately severe course. The piece of skin removed still had the akamushi attached and there was no doubt that it caused the disease. This parasite measured 0.263 x 0.175 mm. and had not as yet sucked much blood.

The development of the bite lesion and its course are as follows. When the akamushi attacks man, it bites firmly into the skin and holds on. There is either no change, at first, or the skin becomes slightly elevated. When this area is touched by the hand, or by clothing, there is a prickling sensation. Even after the mite has been removed this sensation persists whenever the wound is touched. Further changes in the lesion depend upon whether or not the mite be infectious. If not, the bite appears reddened after the removal of the insect, slightly elevated into a small papule, and a brown crust forms at its apex, surrounded by a dark red area. If the akamushi be infectious a small pustule forms in the bite within a few days and the surrounding skin becomes diffusely reddened. As there is no pain at all, this may be mistaken for a simple hair-follicle infection, or it may be scratched off. The pustule dries and forms a crust, or it may remain as such until the onset of the illness.

If these lesions be observed at this time, light reddish elevations 0.8 - 1.5 mm. are seen. Their margin is usually diffuse and at the center there is a yellowish-gray, or orange-brown crust about 2 - 3 mm. in diameter. It may be semi-transparent and honey-colored, with a slightly indented surface. The surrounding area of erythema may not be at all indurated, or it may be slightly so. There is no pain, but tenderness may be elicited and if the pustule be squeezed, the crust enlarges with the progress of the illness and attains its maximum size at the end of the fastigium. The brown color crust is broken and a syrupy purulent secretion escapes. This deepens to dark-brown, or sooty-brown. When the bodily warmth softens the crust, it drops off, exposing an ulcerated area of about 6 - 8 mm. in diameter. It may be as large as one, or even 2 cm. in diameter, is usually round, but may be elliptical; its base extends into the subcutaneous tissue, and its margins are punched-out and sharp. The ulcerated surface is moist and, at first, contains grayish-yellow sloughs;

but it becomes and is soon covered with a serous or purulent secretion which may form a thin crust as it dries. The surrounding bright red area becomes bluish at this time, the skin loses its normal wrinkles and becomes smooth and shiny. When the fastigium is over, there is desquamation and during the defervescent and convalescent stages granulation tissue appears over the base of the ulcer, covered by a hard dark-brown crust, and finally forms scar tissue.

When there are more than two bites they usually have the same appearance, but one may be smaller than the other at any given period. The bite lesion of a mild case, or one of a case of second attack, is similar to the typical wound, but often there may be atypical wounds in second-attack cases. In these the crust is not flat, but sharply acuminate. There may also be atypical bite lesions in cases where these are multiple.

d. Lymphnodes.

Tenderness and swelling is one of the cardinal symptoms of tsutsugamushi disease, other investigators have recognized this as well as the fact that there is also tenderness and swelling of the systemic lymphnodes. We carefully examined the nodes in ninety cases. In many instances swelling of the regional nodes occurs during the incubation period; it is usually discovered by patients who have found an akamushi-bite and have learned to look farther for the swelling. In our histories we found nine, in which the patients themselves discovered the regional lymphatic tenderness and swelling; in five, one or two days, in four three or four days before the onset of tsutsugamushi disease. We therefore believe that these nodes become swollen and tender at the middle of the incubation period.

At the time of onset they are enlarged to 5 x 7 mm., 1 x 1.5 cm. or even 4 x 5 cm.; and are hard. There is tenderness without pain. The lymphnodes nearest the bite become the most swollen, tender and indurated, the tenderness being very acute in some of them and a slight touch evoking shrieks of pain. The swollen nodes remain discrete. Case No. 4 had a bite over the left gastrocnemius and examination of the left femoral nodes, removed three days after onset, showed the

lowermost node to be 5 x 3 cm. This one had been very tender. As one proceeded upward the size of the nodes gradually decreased, as had the tenderness, and the uppermost was 6 mm. in diameter. It had been insensitive. There were no internodal adhesions, but all of the nodes were indurated, the induration being most marked in those that had been most tender. The skin was not adherent to the nodes, but when they were superficially situated, as in the inguinal region, the overlying skin became slightly reddened. There is nothing to indicate the presence of lymphangitis between the wound and the nodes draining it.

Swelling of regional nodes continues till the fastigium and tenderness is severe up to that time, or thereabout, but it gradually decreases and finally disappears before defervescence. Induration also gradually disappears in the same way, the swelling also subsiding in the convalescent stage.

The degree of swelling and tenderness in lymphnodes does not necessarily correspond to the severity of the disease, there may be mild symptoms in a severe case and vice versa.

Swelling of the systemic nodes is not noted during the first day or two, but it gradually becomes marked and is at a maximum during the fastigium. The nodes vary in size from that of a bean to that of a pigeon's egg and are somewhat indurated. This is most noticeable in the case of the cervical or axillary groups, which may exhibit some tenderness that sets in co-incidentally with the swelling and begins to subside during defervescence, disappearing during convalescence. The submaxillary nodes are often much swollen and are tender, which is due to complicating stomatitis and pharyngitis. Such complications, if purulent, may influence the fever-curve. The lymphoid manifestations in cases of second attack, or in atypical cases, are somewhat like those just described, but tenderness of the regional nodes is usually less marked and of shorter duration, while the swelling of the systemic may also be slight.

e. The Eruption.

This in another important sign of this disease; all the investigators have paid it due attention. Bälz and Tanaka have described it, and I have noted it in all of my ninety typical

cases, excepting in a single very mild one. It is divisible into an exanthem and an enanthem.

a. Exanthem: A few scattered lesions about the size of a pinhead appear at first on the trunk and upper extremities in the form of slightly elevated papules with a light pink color. At about this time, or a day later, they also appear on the cheeks and forehead. In time the papules increase in number and size, becoming salmon-colored, their size varying from that of a pin-head to that of the tip of the index finger and their appearance exactly resembling that of the rash in measles. Some of them may be as large as 1.0 - 1.2 cm. They are circular, or irregular and slightly elevated, their margin usually vague, their distribution discrete or grouped, but not confluent. The pink color disappears on pressure, but the red color deepens to dark-red or coppery and gradually fades to a brownish stain and then disappears. Some of the lesions may be macular, rather than papular.

The rash reaches its maximum in three to five days and then gradually fades. Some of the lesions may be acuminate, forming transparent vesicles which later become crusts. I have not seen a single case in which the eruption became hemorrhagic, as noted by Tanaka. The rash is neither itching nor tender and appears almost everywhere, even on the palms, soles, or scalp. It may be found equally distributed over the flexor and extensor surfaces of the extremities, or may be more extensive on the latter. Comparatively few lesions are found around the mouth, wrists, feet, elbow, and patella.

In two pregnant cases (25 and 27 years old respectively) and in one man (30 year old, No. 4), the eruption on the forearm became hemorrhagic when the arm was bound with a rubber tourniquet while drawing blood at the elbow. We have not had this experience in any other instance. In another case (No. 3, 20 year old male) there were petechiae resembling faded spots of the rash during the latter part of defervescence and blood-cultures from this patient were negative. This purpura subsided in two or three days, during which time there was gingival bleeding.

The date of the eruption's appearance, observed in thirty cases, follows:

Number of days after onset	Number of cases
Second day	3
Third day	7
Fourth day	6
Fifth day	11
Sixth day	2
Seventh day	3

In the majority of cases the eruption appears between the third and fifth days, usually on the fifth; there are a few instances where it appeared as early as the second or as late as the seventh days.

Its duration in twenty-two cases is as follows:

5 days	2 cases
6 days	4 cases
7 days	4 cases
8 days	8 cases
9 days	3 cases
10, 12, and 14 days	1 case each

Thus; in the majority of instances, the eruption lasts from five to nine days and in most cases exactly eight. In the latter half of the fastigium, or the beginning of defervescence, the eruption may fade either gradually or rapidly and disappear completely without leaving a trace; or it may leave brownish spots or crusts. In some patients, after it has disappeared completely, small elevations may be palpable for several days. The crusts are yellowish or dark brownish, dot-like, discoid, or flaky, and about 2 to 3 mm. in diameter. The degree to which crusts form varies with each case, in some there may be none at all, in others a large number may form. I had one case in a 12-year-old girl, in whom there were an exceedingly large number of crusts on the forearms and legs. The brownish spots may remain for several days after the subsidence of the fever. Flaky exfoliation may be noted on the surface of such pigmented areas and these may be found on all the extremities. Occasionally they may be transformed into purulent vesicles.

When the rash of Case 3 was examined under a skin microscope, on the third day, the capillaries were found to be greatly distended near the papules, with irregular grayish-white dot-like masses, the centers of which were black. On

the seventh day they were still distended in places and dirty grayish white material was massed in their lumina.

The eruption is absent in atypical cases and in second attacks, in only one of the nine atypical cases was there a rash, and this was limited to a few areas on the face, back, chest, and extensor surfaces of the legs, disappearing after one day, of twelve cases of second attack six had none, two an indistinct rash, and three a slight one. Two of these had an eruption on the fourth and on the eighth days respectively, but it disappeared after three days; in a third case it appeared on the fifth day and increased markedly for five days on the chest, thigh and legs.

b. Enanthem: I have observed eruptions on the soft and hard palate and occasionally on the buccal mucosa. The examination of seventeen cases demonstrates that this eruption appears one or two days later than the exanthem, being noted on the seventh and eighth days in the majority of instances. The enanthem may be seen on the reddened mucosa as redder spots, from the size of a pin head to that of a hat-pin head. A papule may contain several yellowish-white dot-like masses which later slough out to form ulcers. These are not seen in the atypical cases, nor the second attacks.

f. Respiratory System.

The nasal mucosa may be congested coincidentally with the conjunctivae. Epistaxis is frequently found, as already noted by Bälz. It is occasionally very severe and often met with in the fastigial stage. Pharyngitis is sometimes seen in moderate or severe cases. There is often a dry cough.

More than half the cases have bronchial symptoms, the respiratory sounds are rough, with piping rhonchi, or sometimes moist or dry râles, often diffuse, but occasionally limited to the bases posteriorly. There may be muco-purulent sputum, a microscopic examination of which will often disclose many Fränkel's diplococci. In a few cases there may be dullness over the posterior bases, with signs of pneumonia. This is often encountered in autumnal cases. Symptoms of bronchitis are found in mild cases and in second attacks. When pneumonia exists, it involves both lower lobes. Whistling or bubbling râles may be heard. The sputum is thick

and yellowish, or it may be reddish-yellow. Fränkel's diplococci are found and may be identified either by the microscope, by cultural methods, or animal inoculation.

g. Alimentary Tract and Spleen.

In the fastigium the lips are dry, cracked and bleeding, the gums are sore or less reddened and swollen and in a few severe cases they bled and were covered with dark-red coagulated blood. The tongue is at first moist, smooth and not coated, but in the fastigium it becomes grayish-yellow or grayish-brown with sordes. In a severe case it becomes dry and dark brown. When it is moist, mildness of the attack is indicated; when dry, severity. In a severe case thrush-like patches may be seen on the mucosa of the palate, cheek and gums. The mouth is often foul smelling. Congestion of the mucous membrane of the pharynx and palate is always seen in this disease, coming on by the third or fourth day after onset and becoming dark red in the fastigial stage. The patient may complain of pain on swallowing. The congestion gradually fades at the end of the fastigium and the mucosa returns to normal by the latter half of defervescence. The tonsils may be slightly swollen in some cases.

Constipation is usually the rule and is an important symptom in the digestive disturbances. It is even seen in mild or atypical cases. It may be present from the outset, but it often dates from the second or third day of the disease. Until then, stools may be either normal or softish. In a few cases constipation may set in during the fastigium. It lasts throughout the entire course of the disease, but may give way to diarrhea, which is often noted during defervescence or convalescence.

In many cases the abdomen may be distended with gas during the fastigial stage and the patient may complain of nausea, or vomit, during the stage of invasion.

Bälz notes that the liver is only exceptionally enlarged, but we found that it often is, and is felt two finger-breadths below the costal margin, gradually increasing in size during the fastigium and becoming hard and often tender. It may still be enlarged during convalescence. In none of my cases was there icterus.

Spleen: It is well known that the spleen is enlarged in this disease. It was enlarged in thirty-one (54.4%) of fifty-seven typical cases of first attack. The enlargement is usually noted six to ten, occasionally four to five days after onset. It is felt two or three finger-breadths below the costal margin and is as hard as a malarial though not as soft as the typhoid spleen. It is largest in the latter half of the fastigium and gradually diminishes. Occasionally it is found still swollen after two or three weeks, in the convalescent period. In all these cases there was no malaria. In atypical cases of tsutsugamushi disease there is no hepatic or splenic enlargement, but this may be seen in cases of second attack.

h. Nervous System.

According to Bälz and Tanaka, headaches and general hyperaesthesia are characteristics of this disease, the mental condition may be clouded and the patient may have a talkative delirium, or there may be maniacal attacks. Bälz noted that when hyperesthesia is severe, it is found not only in the skin, but also in the muscles; when the muscles of the arm or leg are grasped the patient complains of pain.

Most of our cases complained of headache or a heavy feeling during the early stages. The face has a dull and heavy expression, consciousness is usually clear, there is more or less excitement from the outset and the patient is very sensitive. Sleeplessness is complained of in severe cases and the mentality becomes clouded, the patient talking deliriously. Maniacal excitement is seen only in very severe attacks, with a bad prognosis. During defervescence many patients complain of drowsiness. The speech is always clear. In severe cases there may be pain or rigidity in the neck and nausea and vomiting may occur. Occasionally during unconsciousness the urine may be voided involuntarily.

In the fastigium, muscular pain may be complained of when the muscles are grasped, or lightly touched with the stethoscope. It is particularly noticeable in the muscles of the arm and chest. There may be tenderness to tapping in the tendons, especially the patellar and Achilles tendons. In the fastigium one notes periosteal pain in the tibia when it is

pressed upon with the fingers or tapped with the stethoscope; this tenderness lasts until the latter half of the stage of defervescence.

In severe cases here may be continuous or convulsive tremors of the lips, fingers, and tongue.

The patellar reflex becomes weak in the fastigium and much diminished during defervescence, or it may disappear entirely at this period, gradually reappearing by the latter half of the third or fourth periods, when it becomes exaggerated. The Achilles behaves the same as the patellar reflex, but its recovery is slower. The ankle clonus is very interesting in connection with the changes in these other reflexes, it appears at the time when they begin to be exaggerated or in the latter half of the convalescent stage. As a rule it is noted in severe cases, but there are exceptions and it may appear in the mild ones.

i. Sensory Organs.

Eye: Hyperemia of the palpebral and bulbar conjunctive is always noted. It is first seen in the palpebral portion, extending later to the bulbar and it begins about three to five days after the onset. At first it is bright red, later darkening. At first only an injection of the scleral capillaries, it later becomes diffuse and is at its height in the middle of the fastigium, gradually fading and disappearing during defervescence or convalescence. Photophobia is occasionally encountered and lachrymation is also complained of by a few patients. These symptoms are also seen in atypical cases or in second attacks, but they are usually very slight.

Ear: Deafness occurs in many cases, hearing becoming impaired eight to ten days after onset, which is in the first half of the fastigium, but it may be complained of as early as four to seven days, rarely as late as eleven to fifteen days after the onset. Tinnitus may preceed deafness and is at first slight later becoming more marked. Sometimes a patient complains of feeling as though the external auditory tract had been plugged up, but earache is not complained of. There are no objective changes. This deafness may continue for several days and may often grow very severe, but it gradually lessens and disappears in the defervescent or convalescent per-

iods. It may last for ten days or more, but recovery is always complete; therefore, as Bälz has said, it is not organic, but of nervous origin. It is not usually found in atypical cases or in second attacks, but it may be found in the latter if they be severe.

Skin: We have already stated that edema is found in this disease in the face, eyelids, lower extremities, and soles of the feet; also, that there is never icterus.

The face is flushed when the conjunctivae are: from three to four days after onset and during the fastigium the entire face becomes scarlet, the flush lasting through the fastigium and gradually fading during defervescence to become pale in the stage of convalescence. The skin, as a whole, is hot, flushed, and perspiring; wherefore a peculiar odor is noticeable in the sick-room. There is less sweating, however, in very severe cases. Miliaria and sudamina may be found in the fastigium over the chest and abdomen at first, then spreading over the entire body, and lasting until the defervescent stage. The sudamina may suppurate and become pustules. Decubitus may be seen during the latter half of the fastigium in very severe cases.

j. Urine.

Reports have been made on the urine in this disease, by Tanaka and Hayashi. According to Tanaka it is strongly acid, decreased in quantity, and its specific gravity lies between 1015 and 1030. Albuminuria is found in most cases in the later stages of the disease, but is very slight when measured by the Esbach albuminometer. Red and white blood corpuscles, epithelium, and many granular casts are seen, diazo reaction was positive during the invasion and fastigium, but there was no increased indican output, Leucin and tyrosin were not found. Hayashi reports that the diazo and albumin reactions are almost always positive in severe cases disappearing as the illness subsides. Sugar and bilirubin are said to be absent.

We have examined the urine of seventy-one cases throughout the disease, and not only were the general characteristics of the urine noted, but the diazo, albumin and indican reactions, as well as microscopic examinations for formed ele-

ments, were carefully performed. In a few cases, quantitative as well as qualitative examinations were made, in order to evaluate the metabolic changes in these patients.

(i). General Characteristics of the Urine.

(1) Reaction: At first this is strongly acid, in the fastigium it becomes either weakly acid or almost neutral, then returns to normal. It is not strongly acid as Tanaka has claimed.

(2) Color: In mild, or early cases the color is light yellow, but in severe cases it deepens and becomes dark amber or Burgundy red, as in hematuria.

(3) Specific Gravity: The specific gravity at 15° C is increased as the color deepens and in most cases is between 1033 and 1036, in a few between 1025 and 1029, the highest reading we recorded was 1044.

(4) Quantity: This is decreased and the daily output is, in many instances, between 500 and 800 c. c., in not a few from 300 to 400, or as low as 200 to 250 c. c. per day.

(ii). Qualitative Examination.

(1) Diazo reaction: This was positive in all cases, excepting the majority of atypical and the second attacks. This reaction is in close relationship to the appearance of the eruption as in other eruptive diseases; therefore, even in severe cases, whenever there is little eruption, the reaction will be weak. In second attacks it was negative, even in severe cases, because the eruption was very slight. The results of thirty-two observations as to the time of appearance of the diazo-reaction follows:

Coincident with eruption	7 cases
A day after its appearance	12 cases
Two days after its appearance	3 cases
Three days after its appearance	3 cases
Four days after its appearance	3 cases
Five days after its appearance	3 cases

Thus the day after the appearance of the eruption is the time when this reaction first becomes positive in most instances. How long it continues positive depends upon the severity and extent of the eruption. The following figures give its duration in twenty-seven cases.

Days duration	Number of cases
1	1
2	1
3	2
6	1
7	2
8	5
9	1
10	3
11	4
12	4
13	1
14	2
17	1

The reaction, therefore, lasted from eight to twelve days in most cases. In mild and in very severe cases which proved fatal, it lasted from one to three days. The disappearance of the reaction is in close relationship to that of the rash, but the former lasts longer. The following are the results of the observation of nineteen cases.

Diazo reaction disappeared:

Number of days after disappearance of the rash	Number of cases
1 day	5
2 days	2
3 days	6
4 days	1
5 days	2
6 days	1
7 days	1
8 days	1

Thus the majority of cases give a negative reaction one to three days after the rash has gone, but in a few cases it remained positive for about a week. This reaction is seen in the fastigium and defervescent period, but there was no case in which a positive reaction lasted throughout convalescence.

(2) Indican reaction: In four cases out of forty-six the indican reaction was negative, in two cases faint, but in the rest it was positive. There were six strongly positive, eight moderately so, the rest being weakly positive. The intensity of the reaction was, however, not parallel with the severity of the disease and in each case this intensity fluctuated greatly during the course of the illness, sometimes running a highly irregular course. It continued positive for a day or two in a few cases, in many for several days, and there are a few in which it remained so for ten or more days. Atypical cases,

and second attacks, gave no positive indican tests, with a few exceptions.

(3) Albumin reactions: Albumin was found in all the typical cases; there were twenty positive tests with sulphosalicylic acid, twelve with this and Heller's tests, and nineteen with the heat test. The urine of all the severe cases was positive only with the heat test. There were only seven cases in which there was albumin enough to be measured with Esbach's albuminometer, the amount being between 0.2 and 1.0%, rarely 2.5%. The albumin output, like the diazo reaction, is very irregular and it is most marked in the fastigium and defervescent periods. It usually disappears in the latter half of defervescence or during convalescence, but may last longer in some cases particularly if they be complicated by other diseases.

Albumin is not found in atypical cases. In second attacks it may be demonstrated with sulpho-salicylic acid or Heller's reagent; occasionally it may be found with the heat test.

(4) Urochromogen and Urobilin: These reactions were tested in fifteen or more cases. The urochromogen appears about the same time as the diazo reaction in typical, but not in atypical cases. It persists longer than that reaction, about six to sixteen days, but its intensity does not necessarily correspond to that of the diazo reaction. The urobilin test, compared with the urochromogen, is weaker and more transitory.

(5) Pathological formed elements: These were found in forty-six out of fifty-three cases; leukocytes, kidney epithelium, and various types of cast. Bacteriuria was noted in thirteen cases. Formed elements were found in some instances of second attack.

(iii). Quantitative Studies.

In order to form some idea of the metabolic changes in patients suffering from tsutsugamushi disease, samples of urine were kept for analysis, with a few drops of toluol, in brown bottles. The samples were taken from a day's output of seven patients during the fastigium. For the estimation of sodium chloride, the Volhard-Salkowski method; for phosphates, the uranium solution method; and for nitrogen deter-

mination, the Kjeldahl method were used. These estimations were made under the direction of Professor Kawakita, to whom our thanks are due. The results were as follows:

Case No.	Age	Day of taking sample after onset	Chlorides gms.	Phosphates gms.	Total Nitrogen gms.	Urea gms.	Sp. Gr.	Amt. per diem (c.c.)
1	25	7	4.560	0.730	8.690	18.32	1025	820
2	14	9	4.280	1.080	10.400	22.29	1031	530
3	34	6	7.800	1.496	16.445	33.62	1035	900
4	34	12	1.850	1.342	13.400	28.72	1028	750
5	13	11	4.902	0.939	14,935	31.96	1035	700
6	30	10	6.300	1.024	14.770	31.65	1027	700
7	19	8	4.500	1.125	10.051	21.54	1036	650
8	17	Convalescence	5.280	0.269	2.910	6.24	1015	930
Normal averages			18.960	1.553	12.270	24.15	1019	1344

As will be seen from the above table, the chlorides are very much decreased in comparison with those of normal Japanese. They are reduced to one-third, a quarter, or even one-tenth of normal, the chief cause for this being the decreased intake of chlorides, owing to reduced appetite. The output of phosphates in cases 3, 4 and 6 (all adult farmers) was from 1.496 to 1.041, which is slightly less than the average normal execretion. However, cases 2, 5, and 7, who were boys, had about the same phosphate excretion as did the adults, so that this disease has no influence on the excretion of phosphates. In some cases the output is increased. It is said that the phosphates in urine are derived from the food, and if this be so the output must be decreased, as these patients eat very little. It may be that the alkali earths and alkaline in food, or the decomposition of nuclei following the destruction of the leukocytes, may have something to do with the excretion of phosphates. Nitrogen in the urine is very much increased; there was one case in which it reached 16.-445, which corresponds to 33.62 urea. There were not a few cases in which the urea output was over 30.00, which indicates how vigorously the process of protein-splitting goes on in this disease.

k. Decrease in Body Weight.

Since this disease runs a very rapid course, the emaciation of the patient is very marked. The following case illustrates this point: a young man of twenty, Case 3, had a severe type

of the desease; on the seventh day after onset he weighed 55.67 kgm. and on the thirty-sixth 41.9 kgm., having lost 13.77 kgm. or about one-quarter of his body-weight.

4. Complications.

Bronchitis was seen in over half of the fifty-eight cases that ran a typical course. Five had complicating pneumonia, three suppurative submaxillary lymphadenitis with abscess, and one of these had gangrenous stomatitis and parotitis as well. Five pregnant women all aborted, three during the stage of defervescence and two during convalescence; in all of these, however, the prognosis as to the disease was good.

5. Number of Cases and the Death-Rate.

It has been compulsory since 1903 for attending physicians to report cases of tsutsugamushi disease and their outcome, in Niigata prefecture. The following table shows the number of cases reported yearly:

Year	Number of cases	Number of deaths	Death rate per 100
1903	217	56	25.6
1904	278	89	32.0
1905	125	29	23.2
1906	195	57	27.7
1907	254	72	28.3
1908	282	79	30.1
1909	192	52	27.1
1910	175	48	27.4
1911	47	12	25.6
1912	71	26	36.6
1913	94	32	34.0
1914	174	57	32.6
1915	224	78	34.8
1916	209	64	30.6
1917	227	94	41.4
1918	212	86	40.5
1919	73	25	34.1
1920	131	46	35.1

The total number of patients in eighteen years was 3,166 with 996 deaths, the death-rate being 31,5%; the highest mortality in any one year was 41.4% in 1917, the lowest 23.3% in 1905. In Akita and Yamagata prefectures the number of cases of tsutsugamushi disease is far less than in Niigata prefecture, but the death rate is rather high. The following is a table showing the number of cases in Yamagata prefecture and their outcome between 1913 and 1917.

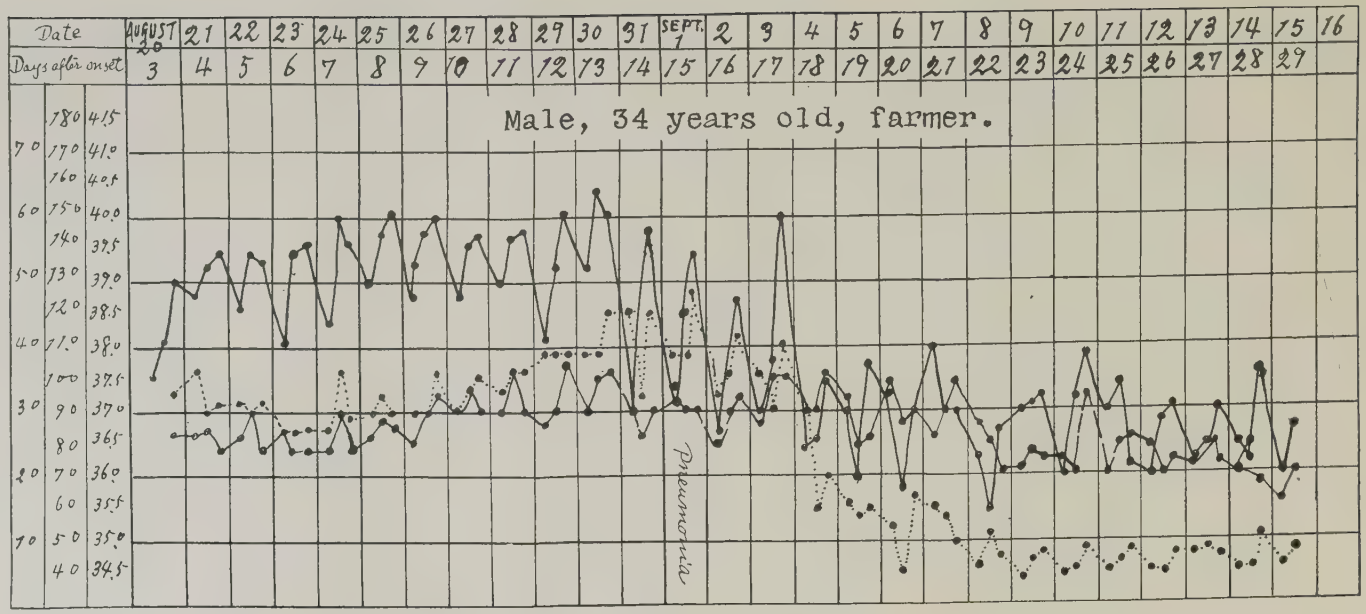


Figure 9

Year	Number of cases	Number of deaths	Death rate per 100
1913	39	24	61.4
1914	19	12	63.1
1915	22	10	45.5
1916	11	6	54.5
1917	20	10	50.0

The average death rate is, in this case, 55.8% during the five-year period.

There are no official statistics of this disease in Akita prefecture, so that accurate data are unobtainable, but according to Tanaka, who has been studying the disease there since 1890, the data for the three years given below are as follows:

Year	Number of cases	Number of deaths	Death rate per 100
1915	33	12	36.3
1916	39	13	33.6
1917	61	24	29.3

Comparison of the above tables demonstrates that the death-rate is highest in Yamagata prefecture and lowest in Niigata; but the number of cases is Niigata is far greater than in the other two prefectures. The low death rate in Niigata can not be summarily attributed to the low virulence of the disease in that prefecture. Generally, then, the death rate is between 30% and 40%. These death rates apply to primary cases and those of second attack. Strictly speaking these should be kept separate, for the death-rate among cases of second attack is very low.

The following two curves were made from 1,637 cases which occurred during eleven years, inclusive of 1910 and 1920. Curve 1 (Fig. 10) shows the death rate of males and females according to age and curve 11 (Fig. 10) shows the percentage of males and females of each age. (Solid lines - male, dotted - female.)

The increase in age and that in the incidence of the disease is parallel, the highest number of cases occurring between 21 and 20 years, males being more commonly attacked than females. On the other hand, the death rate is higher among females than among males and the relation of sex to mortality is more marked during the period of childhood, 6 to 15 years. During this period females appear to be less resistent than males, but from then onward, to 30 years, there is little dif-

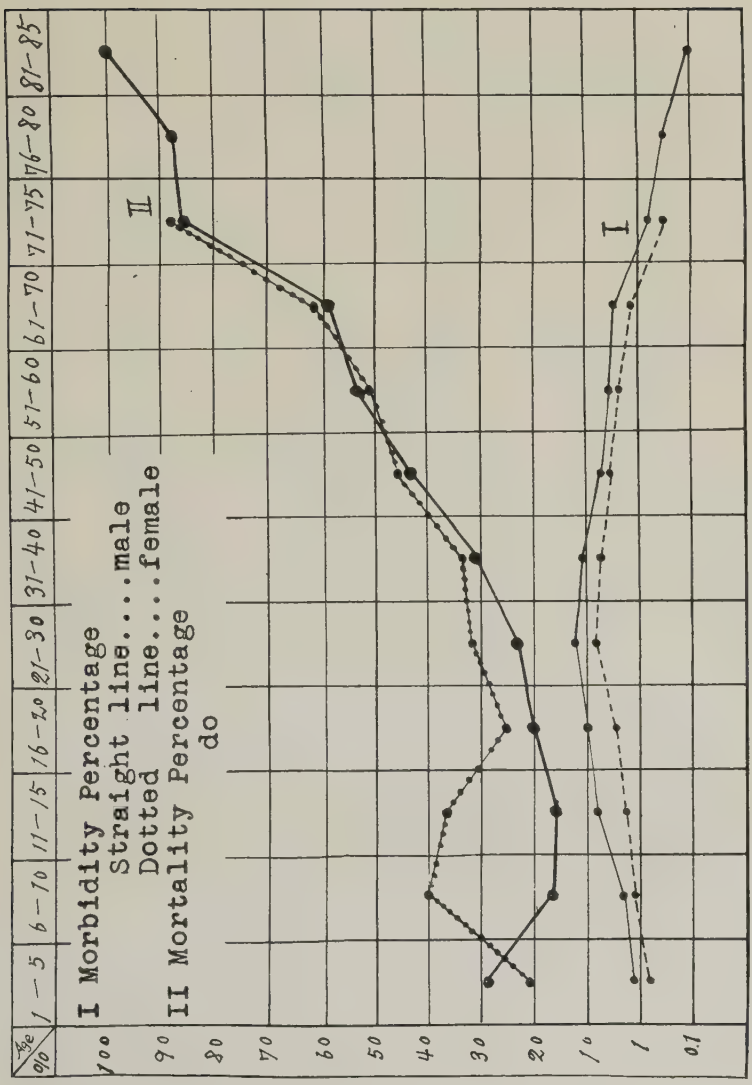

Age
%
1 — 5
6 — 10
11 — 15
16 — 20
21 — 30
31 — 40
41 — 50
51 — 60
61 — 70
71 — 75
76 — 80
81 — 85
100
90
80
70
60
50
40
30
20
10
1
0.1
I Morbidity Percentage
Straight line....male
Dotted line....female
II Mortality Percentage
do
II
I

Figure 10

ference between the sexes. After thirty the mortality-curve rises very sharply.

Of the ninety hospital cases in our series, seventy-nine represented first attacks, and eleven the second or third. There were no deaths among the latter group. The following table shows an analysis of these ninety cases.

Age	Sex	Number of cases	% of series	Total	Number of deaths	by age	Total deaths
1 - 5	M	1	1.26		0	0	
	F	1	1.26	2	0	0	0
6 - 10	M	3	3.79		1	33.33	
	F	1	1.26	4	1	100.00	2
11 - 15	M	7	8.86		1	14.28	
	F	4	5.06	11	0	0	1
16 - 20	M	9	11.39		0	0	
	F	1	1.26	10	0	0	0
21 - 30	M	13	16.45		1	7.69	
	F	9	11.39	22	0	0	1
31 - 40	M	4	15.08		0	0	
	F	5	6.32	9	2	40.00	2
41 - 50	M	7	8.86		3	42.85	
	F	6	7.59	13	3	50.85	6
51 - 60	M	4	5.06		1	25.00	
	F	1	1.26	5	1	100.00	2
61 - 70	M	2	2.53		2	100.00	
	F	1	1.26	3	1	100.00	3
Total	M	50	63.26		9	18.00	
	F	29	36.66	79	18	27.58	17
Total M and F		79	99.92		17	21.50	

Age	Sex	Number of cases of 2nd attack	Total	Number of cases of 3rd attack
2 - 15	M	1		
16 - 20	M	1		
21 - 20	M	3		
31 - 40	M	2		
41 - 50	M	1		1
51 - 60	M	0		1
61 - 70	M	1		
Total all ages		9		2
Grand total			11	

As the above tables demonstrate, more than half of the seventy-nine primary infections occurred during the period between eleven and thirty years; the death rate for both males and females is 21.5%, for males alone 18%, and for females 27.58. These death rates are far lower than those reported in Niigata prefecture at large, as the curves show, the lowest death rate is found between the ages of eleven and thirty years, there were only two deaths (4.76%) out of thirty-four

cases of this age. After thirty years of age the rate is much increased and after forty more than half the cases die.

The low death rate in our series, compared of that reported from the prefecture at large, is probably due to the favorable symptomatic treatment and the good nursing in the hospital, as there is no specific treatment. Out of the seventeen fatal cases one had complicating gangrenous stomatitis, parotitis, and submaxillary lymphadenitis; another died of uncontrollable hemorrhage as already described. The other fifteen fatal cases had no complications and died from the effect of the toxin of tsutsugamushi on the heart.

6. Contraction of the Disease.

The disease is contracted by those who inhabit or come into the endemic regions, or by those who handle the vegetables, grain, hay, wood and other products of the district. Consequently most of the patients are farmers, young adults, for these have more opportunity to come into contact with the mites that transmit the disease. Those who have not been in the endemic region may contract the disease by contact with various products thereof. Fishermen are equally exposed to the disease, but unless they go naked, offer less opportunity for the akamushi to bite them. We have four cases of fishermen in our series of ninety patients, and one case of a man had merely passed through an endemic region. To recapitulate: exposure to infected akamushi, either by entering an endemic region or coming into contact with its products, is the cause of one's contracting the disease.

7. Relation of Seasons to the Occurrence of Tsutsugamushi Disease.

The disease is most frequently contracted during the summer. Statistics collected in Niigata prefecture during the past eleven years show that it is most prevalent during July and August, less so in June, September, and October. It occurs as early as the latter part of May and rarely as late as December. The dangerous season is from the last of June to the first part of October. The following table (Figs. 11 & 12) shows the temperature curve throughout 1919 and the occur-

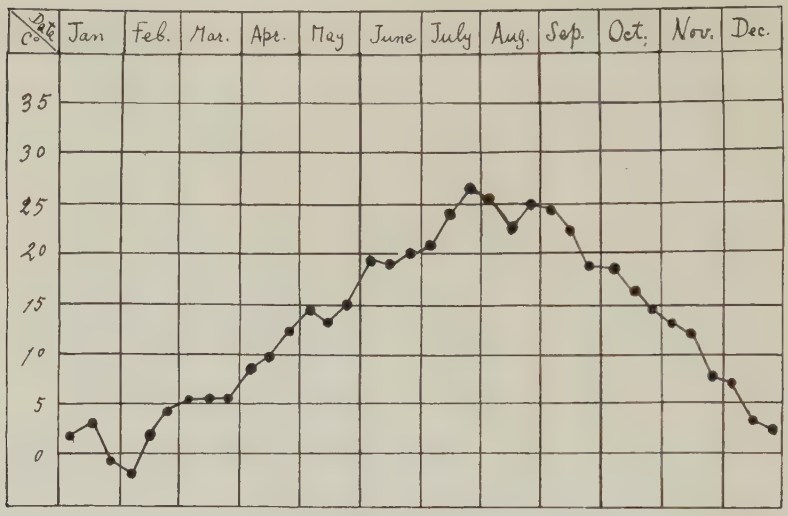


Figure 11
Temperatures in 1919 in Niigata meteorological observatory

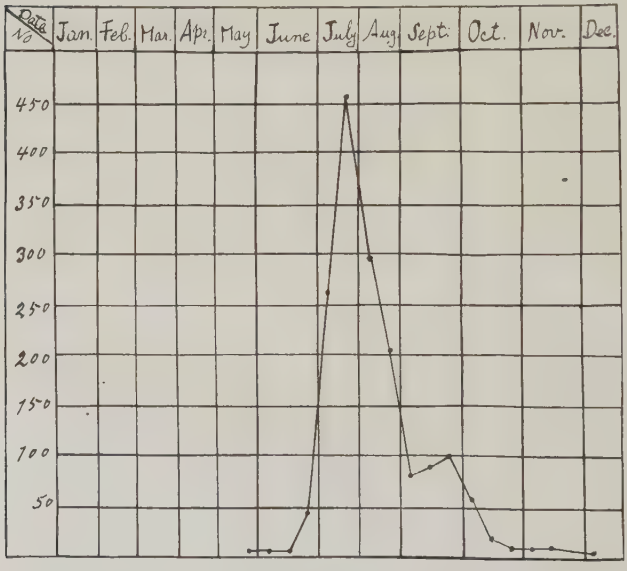


Figure 12
Monthly distribution of patients during 11 years of Niigata prefecture, total 1,650 cases

rence of cases in each month during the last eleven years in Niigata prefecture, they are prevalent during the latter part of July, when the weather is warmest. According to Hatori, the same relationship exists in Formosa, where the disease occurs from April to November and is most prevalent during June and July, when the weather is warmest.

8. Immunity to Tsutsugamushi Disease.

One attack confers some immunity, but it is not absolute, as one may contract the disease again. The second attack is usually very mild. There were twelve out of our ninety cases who had more than one attack.

Case No.	Age	Years between 1st and 2nd attacks	Years between 2nd and 3rd attacks
1	27	3	
2	53	21	19
3	22	8	
4	41	26	11
5	65	37	
6	11	7	
7	34	15	
8	29	16	
9	48	26	
10	21	2	
11	18	8	
12	21	1	

There were ten out of twelve cases that had two attacks and two having three attacks. Four cases showed an interval of over ten years between the first and second attacks, the longest being thirty-seven years. Both cases of three seizures had an interval of more than ten years between the second and third attacks. We observed Case 12 in both his attacks, the first in 1916. He was bitten at the inner canthus of the right eye and the course of the disease was very severe. The next year he was bitten on the mesial aspect of the right thigh and had rash and markd leukopenia, but the course of his illness was very mild. (Fig. 13)

In 1917 we investigated a certain locality in an endemic region and found thirty-three cases, three of which had had three, the rest two attacks. The longest interval between seizures was about 25 - 35 years, but there were sixteen victims (48.6%) who had subsequent attacks within ten years

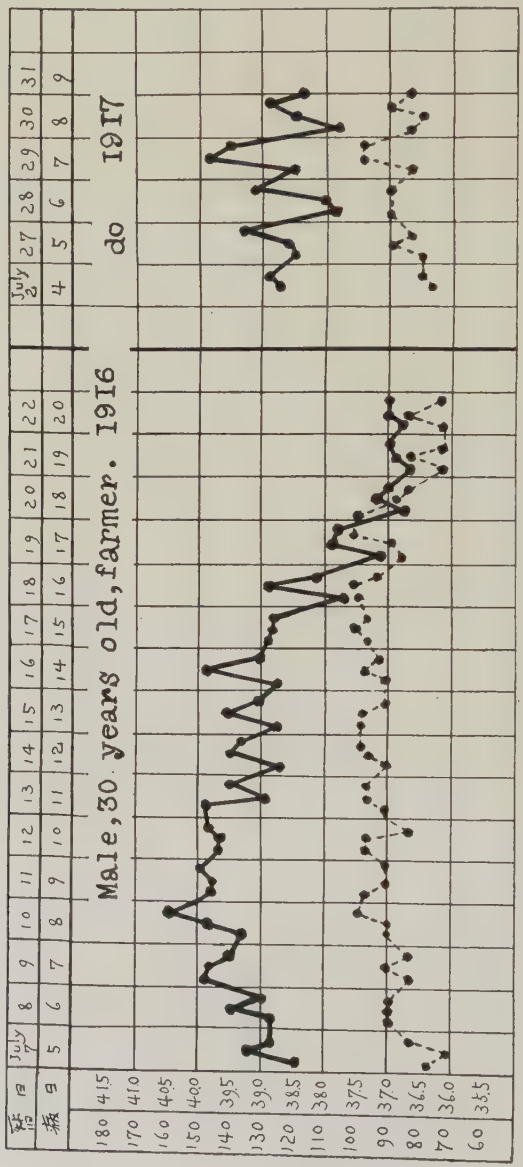


Figure 13

after the first one. Seven of these had an attack the following year.

All this indicates that more than half of the patients suffered the second attack within ten years, some of them the year following. The immunity bestowed upon the victim of one attack of this disease is, therefore, not very marked as compared with that of other exanthemata such as small-pox, or measles. An attack confers some immunity, however, as the next one is usually very mild. There are, however, exceptions; we had a patient of twenty-nine, who had the first attack at thirteen; the second attack was quite severe and he had fever for three weeks, though the rash was scanty.

9. Treatment.

There is no specific treatment. In one case we injected 0.4 gms. of neosalvarsan intravenously and gave quinine salts by mouth in 0.7 - 1.0 gm. doses, to a strong farmer, with no effects. Several injections of convalescent serum, in doses of 4.7 - 9.0 cc. were given to two patients without effect. Therefore, our treatment was limited to symptoms and, avoiding antipyretics, we gave cardiac stimulants and kept the patients absolutely quiet. Other symptoms were treated as the occasion arose. It is needless to say that hospital treatment is far more favorable than home treatment; the death-rate in our series was far lower than those outside and this is due, no doubt, to hospital care.

CHAPTER II.

PATHOLOGICAL STUDIES.

The pathology of this disease is not completely worked out because necropsy material is difficult to obtain; the total number of necropsies performed by Tanaka in Akita prefecture since 1892 is fourteen, with reports of macro- and microscopic observation; in Niigata it is eleven, three necropsies having been done at Nagaoka Hospital in 1877, two by Kawakami (9) in 1878, one by Shibukawa and one by Sawada in 1904, and one by Kuroiwa in 1905. These reports were all merely macroscopic descriptions (excepting Tanaka's). Hayashi (42), however, did three necropsies between 1906 and 1911 and reported histological as well as gross findings.

Since I began the study of tsutsugamushi disease in 1913 I have had the opportunity of doing necropsies on six typical cases and on one in which the patient died during convalescence. Four of these were done on patients who died while being treated in our investigation station. Besides these, there were three miscarriages in pregnant women during the course of the illness and the fetuses were examined.

Case Number	Sex	Age	Days between onset and death	Hours between death and necropsy
1	M	57	about 7	12½
2	M	30	19	5¼
3	F	33	9	5⅓
4	F	22	9	3⅔
5	F	42	about 21	6⅓
6	M	42	11	12½
7	M	48	second week in convalescence	8½

The three fetal cases were as follows:

Case Number	Sex	Time of miscarriage after onset	Body length or weight	Hours post obit.
1	F	8 days	30.5 cm.	5 hours
2	F	15 days	22.0 cm.	10 hours
3	M	12 days	1700.0 gm.	40 minutes

Besides the above material, various organs from patients dying of tsutugamushi disease and necropsied by Hayashi and Kuroiwa were utilized. The specimens of specific pathological changes in the disease, such as skin necrosis, lympha-

denitis, and rash were taken as biopsies from patients during different stages in the course of the disease. In order to study the development of the skin necroses, the area around the bite was removed and examined before the disease was manifest, under local anethesia. Material taken from experimental animals was studied in addition and the results of these studies are included in the section on experimental pathology. All the usual gross and microscopic methods of examination were resorted to. Before giving a detailed description of the findings in the various organs, which will be divided into several sections, I shall present the protocols of the two cases whose clinical records were reported in the previous chapter.

1. Necropsy Findings.

Case I, male, 30, farmer. (Clinical record Case No. 4).

Anatomical Diagnosis. Edema of lungs, acute splenic enlargement, cloudy swelling of viscera, slight enlargement of liver, general lymphadenopathy, necrosis of right femoral lymphnodes, low coagulability of blood, diffuse hypostatic congestion, insect bite over right gastrocnemius.

Body: Is that of an emaciated man 1.55 m. in length. Rigor mortis is marked in the jaw and extremities. There are diffuse dark-purple discolorations in the dependent portions. The skin is moist, but there is no icterus. There are many areas of brownish eruption, a part of which is covered by brownish crusts, on the extremities. The submaxillary, cervical, and both groups of axillary and inguinal lymphnodes are swollen, especially those of the right groin, which are as large as peas and indurated. An operative wound presents in the right femoral region. It is about 3 cm. long, its base is red and granular and covered with yellow or yellowish-gray sloughs.

Primary Incision: from suprasternal notch to mons pubis, reveals very little subcutaneous fat, which is yellowish. The skeletal muscles are light-red and dry. There is no exudate in the abdominal cavity, the great omentum is slightly congested and rolled upwards. The parietal and visceral peritoneum is smooth and shining. The intestines are distended with gas and the intestinal serosa is congested to a moderate degree. The serosa of the cecum exhibits many grayish-red spots about finger-tip size and at their centers one notes marked injection of the veins. The appendix lies in its normal position. The stomach is not congested; the liver is slightly enlarged, its surface smooth, grayish-brown, and hard. The spleen is large and firm, not adherent to the diaphragm. The mesenteric lymphnodes are large and firm, and shot with petechial hemorrhages. The diaphragm is at the third rib right, fourth rib left. No abnormality of ribs or costal cartilages is noted. Upon removing the sternum, the right pleural cavity is found to be obliterated by fibrous adhesions, the left cavity is free. No fluid is found in either. The right lung is somewhat distended. The thymus is vestigial. The large veins contain a large quantity of fluid blood.

Thoracic Organs: The pericardium contains about 30 c. c. of clear, yellowish fluid. The epicardium is smooth and lustrous. The heart

weighs 245 gms. and is contracted to smaller than fist size. There are no petechiae on the epicardium. The right atrium is slightly distended, containing a moderate amount of yellowish, bloody fluid; the left atrium contains a small amount of dark-red fluid blood. The right ventricle is slightly dilated, its endocardium smooth, its myocardium somewhat cloudy. The valves are normal. The left ventricle is normal in size, its endocardium smooth, its myocardium also somewhat opaque, and grayish. Its valves are normal. The intima of the aortic arch is smooth, but two or three grayish-yellow nodules are found near the coronary orifices.

Left Lung: The surface is covered by fibrous adhesions. Its aërated portion is dark-red. this color alternating with paler atelectatic areas. There are many petechiae on the pleura. The cut surface shows much blood with a few hemorrhagic foci. There is nothing to indicate pneumonia or tuberculosis. The bronchial mucosa is much congested, the peribronchial nodes are swollen and dark red.

Right Lung: The surface is smooth and its pleura shows a few petechiae. The upper and middle lobes are emphysematous, the lower dark red in its aërated portions. The cut surface drips blood and there is no indication of consolidation. The bronchi are much congested and the peribronchial nodes are swollen and pigmented.

Abdominal Viscera: Spleen—17 x 11 x 4 cm., 355 gms. The pulp is swollen, granular and dark red on the section-surface, its follicles may be recognized, the organ is firm.

Liver: Right lobe—19 x 17 x8 cm., Left—7 x 12 x 3.5 cm.; 1,470 gms. The surface is smooth and the margins blunt; on section one sees a swollen parenchyma with readily distinguishable grayish-yellow lobules. There is no distention of the bile ducts. The gallbladder is contracted and contains a small quantity of thin yellowish-green bile readily expressible through Vater's papilla. Its mucosa is normal.

Stomach: This contains about 120 c. c. of dirty, dark fluid that smells ammoniacal. The mucosa is grayish-white, congested in patches and covered with dirty shreds. The mucous secretion is increased. The pyloric orifice admits the passage of the index-finger.

Intestines: The duodenum contains a small amount of thin, grayish-yellow, gruel-like material; its mucosa is grayish-white. The rest of the intestine contains liquid feces. The jejeunal mucosa is grayish-white, moderately congested and swollen, its follicles are not much enlarged. The colon contains fecal matter, varying from soft to scyballous. Its mucosa is rather congested. The cecum contains a moderate amount of liquid feces, its mucosa shows many gas vesicles. The appendix is normal. The sigmoid contains much feces. The rectum is much congested.

Pancreas: Soft and congested, and lobulated.

Kidneys: Left—13 x 5 x 4 cm., 170 gms.; Right—11 x 5 x 4, 160 gms. The surface of both kidneys is smooth, the stellate veins distinct. The consistence of the right organ is normal, that of the left slightly soft. The parenchyma is dark red, the cortex slightly swollen and grayish yellow. The pelvic mucosa is slightly congested. There are a few pitted depressions on the surface of the right kidney.

Suprarenal Glands: Each weighs 8 gms. The cortex is not rich in lipins and is grayish yellow.

Organs of Neck: There are two cervical nodes the size of red beans, the left are the size of peas and are hard. The submaxillary nodes are also indurated. The faucial mucosa is slightly congested and that of the esophagus is stained with bile in places. There are no erosions. The thyroid contains a moderate amount of colloid and is reddish-brown.

Other Organs: The aortic intima is smooth and normal. The retroperitoneal nodes are the size of peanuts and slightly indurated. The mediastinal nodes are the same size and firmly adherent. The right inguinal nodes are also of this general size and are hard, their cut surface is granular. The right femoral nodes are the size of walnuts, their section surfaces show necrotic areas surrounded by zones of congestion. The bladder mucosa is pale and the prostate normal. The testicular parenchyma is swollen and the spermatic ducts easily seen. The femoral bone marrow is red.

Case II, female, 32. (Clinical record Case No. 5).

Anatomical Diagnosis. Pulmonary emphysema, subpleural hemorrhages (left), acute splenic enlargement, cloudy swelling of viscera, marked hypostasis, general adenopathy, cerebral hyperemia and edema, insect-bite on left shoulder, ascariasis and ankylostomiasis.

Body: Is that of a well-nourished woman, 1.37 m. in length. Rigor mortis is present. Livores mortis noted on dependent portions of the body. The skin is moist, there is no icterus. There is a faded rash on the upper extremities and many miliaria crystallina in the axillary fossae and on the abdominal skin. Over the left scapular region is a bite-wound covered with a dark brown crust, 7 mm. in diameter and surrounded by an area of desquamation. The cervical nodes show swelling and some induration of the right supraclavicular group, which are as large as peas, the left are not palpable. There is no swelling of the axillary nodes, the inguinal chains are slightly swollen, but soft.

Primary Incision: from suprasternal notch to mons veneris, discloses a fairly well developed panniculus of yellow fat. The skeletal muscle is dark red and dry. No abnormal fluid is found in the abdominal cavity, the omentum is rich in fat and moderately congested. The parietal and visceral peritoneum is smooth and shining, moderately congested. The intestines are slightly distended and their serosa is also slightly congested, but smooth and lustruous. The bladder is moderately congested. The mesentery contains much fat and is congested, its lymphnodes swollen. The appendix is normally situated. The liver is slightly enlarged and bluish-red, the gall-bladder congested and the stomach contracted. The diaphragm is at the fifth rib on both sides. No abnormality of the ribs or costal cartilages noted. The mediastinal nodes are somewhat swollen and congested. The lungs are very voluminous. There is no fluid in the pleural cavities. The thymus has atrophied. The large veins contain fluid, dark-red blood.

Thoracic Organs: Heart: There is no increase in the pericardial fluid, the epicardium is smooth and shining and is slightly congested. The organ weighs 200 gbs., approximates the size of the fist, and is well contracted. There is much fat in the epicardium. The right atrium contains a moderate amount of fluid blood with a small amount of chicken-fat clot. The left atrium contains a small quantity of fluid blood. The right ventricle is not dilated, its valves are normal; the left is the same, and its valves are also normal. The myocardium is somewhat cloudy. The aortic orifice is nodular and grayish yellow.

Right Lung: There is a small amount of fibrous adhesions over the lower portion of the lower lobe. Section surface is bloody. No evidence of consolidation. The bronchial mucosa is congested and covered with foamy fluid, the bronchial nodes are swollen and anthracotic.

Left Lung: This is distended with air and there are many petechial hemorrhages beneath the pleura of the lower lobe. The cut surface is

bloody and there are many superficial hemorrhages in the lower lobe. No consolidation. The bronchial nodes are adherent and swollen, the bronchial mucosa reddened and covered with foam.

Abdominal Viscera: Spleen: 13.5 x 9 x 3.5 cm., 165 gms. The capsule is tense, the cut surface drips blood, the follicles are distinct, the pulp swollen and not easily scraped off.

Liver: 24 x 12.5 x 8.5 cm., 1,070 gms. The capsule is smooth, the margins blunt. There is a small nodular depression over the right lobe. The cut surface drips blood. The parenchyma is cloudy and swollen, its lobulations distinct. There is no distension of the bile ducts, the gall-bladder contains a moderate quantity of thin, yellowish-brown bile, readily expressed through Vater's papilla. The mucosa is stained dark yellowish-brown.

Stomach: The pyloric orifice is patent, the organ contains a small quantity of yellowish-brown fluid and a large number of ascarides. The mucosa at the pylorus is slightly gray and at the fundus yellowish. There is no autodigestion.

Intestines: The duodenum contains a small amount of yellowish-brown fluid and a large number of ascarides. The mucosa is stained yellowish-green. Elsewhere the intestines contain mucus, feces, a few ascarides, and many hook-worms. The mucosa is edematous, its follicles normal. The sigmoid contains a large number of dead ascarides and fecal matter. Its mucosa is slightly congested. The rectum is empty.

Pancreas: This is hard, congested, and distinctly lobulated.

Kidneys: Right—10 x 5 x 3.5 cm., 100 gms. Left—11 x 5 x 4 cm., 120 gms. Their capsules strip readily leaving a smooth surface on which the stellate veins stand out distinctly. There are traces of fetal lobulation. The cut surface shows swelling and cloudiness of the cortex; the medulla is dark red. The pelvic mucosa is congested.

Suprarenal Glands: Right, 5 gms.; Left, 4.5 gms. The cortex is grayish yellow with a distinct pigmented layer and hard consistence.

Ureters: The right ureter is slightly dilated.

Pelvic Organs: The bladder mucosa is slightly congested. The vaginal lining is smooth and the cervix is filled with pale red mucus. The left oviduct is much congested. The right ovary is edematous and congested and contains two corpora lutea. The left ovary contains two corpora lutea. The left ovary contains a cyst the size of a grape and several smaller ones. It is swollen.

Organs of the Neck: The tonsils are slightly swollen but show nothing abnormal on section. The faucial mucosa is slightly edematous and that of the esophagus is bile-stained. The thyroid shows an increased quantity of colloid. There is no abnormality in the submaxillary glands.

Other Organs: The aortic intima is smooth and has a few yellowish gray nodules and corrugations.

Lymphnodes: The left supraclavicular nodes are 3 x 4 cm. in size and indurated, their section surface drips blood. The left cervical nodes are swollen, indurated, and congested. There is no swelling of the right supraclavicular nodes. The right cervical nodes are swollen, but not much indurated. The left axillary nodes are 3 x 4 cm. in size and the right ones about 0.7 cm. in diameter. The right inguinal nodes are swollen but not very much indurated. The left are the same. The left femoral nodes are 0.7 cm. in diameter, the right 1.5 cm., but not very much indurated.

Femoral Bone Marrow: Grayish red.

2. Summary of General Findings in Necropsies.

General Appearance of Subjects: Rigor mortis is marked and there is post portem lividity in dependent portions. There may be patches of discoloration on the chest. The skin is usually moist, there is no icterus. The face may be more or less swollen and cyanotic. Traces of faded papules with crusts may be found at some necropsies. Typical bite-wounds and regional or general lymphoid enlargement is noted. Enlargement of the spleen and cloudy swelling of other organs with decreased coagulability of the blood are always found.

3. Pathology of the Bite Lesions.

As has already been mentioned in the section on clinical studies, the typical bite is caused by the akamushi which carries the virus, although all akamushi do not. The skin necrosis is produced by the bite of an infectious akamushi; if it be non-infectious no local necrotic changes are produced in the skin. Therefore, we may divide these wounds into two groups: simple and infectious.

4. Simple (Non-infectious) Bites.

A. Simple (Non-infectious) Bites.

As material we used either fresh bites which still had the akamushi attached, or wounds which had already become encrusted. Besides human material with attached akamushi, the skin of monkeys and field-rats infested with them, were examined. The encrusted material was carefully guarded to avoid any injury to the tissue before fixation.

Histological Examination: There is not only mechanical injury to the skin, but the akamushi-bite causes other marked changes. The epithelium is depressed and the Malpighian layer becomes necrotic and undergoes colliquation. At the center of this area and attached to its surface, the two sharp palpal claws of the mite are seen penetrating the horny layer. Between these palpae a syphon, or sucking-tube, is thrust into the corium. Surrounding it, with a narrow space intervening, a homogeneous sheath of hyaline substance is observed, which stains purple with hematoxylin, red with Van Gieson's stain. That it is brittle is shown by the fact that it is easily injured

by handling. Atrophic epithelium with dead nuclei and brown pigment is found around this homogenous substance, which may contain the pigment. The nuclei of the epithelium become vesicular.

The superficial layer of corium becomes necrotic and forms a cyst which contains serous fluid, mono- and polymorphonuclear leukocytes. These cells have migrated from the outlying tissues as is shown by the fact that the connective-tissue surrounding the cysts contains many of them, but their number decreases as the distance from the cyst is increased. Only mononuclears are seen in the outlying zones. The surrounding capillaries are more or less distended and surrounded by a small number of vesicular cells which are poor in chromatin and cytoplasm.

The changes just described are not as marked as those produced by the sting of bees or the scorpion, but they do not represent a reaction to mere mechanical injury, being the result of the stimulus of the secretions of the mite. Gudden (36) suspects the existence of a lytic venom in *Leptus autumnalis,* which closely resembles the akamushi, and believes this to be the means of dissolving the epithelium of the host. Tanaka removed an akamushi which had bitten a man and, after emulsification, injected it into another individual; in other cases he merely crushed the mite while on the skin, leaving it in situ. In both instances he obtained typical reactions similar to those seen in the natural insect-bite. These facts indicate that the mite secretes some toxic chemical substance. The akamushi has far larger salivary glands than those described in *Trombidium fuliginosum* by Henking. The secretion of these glands, together with those of the stomach and liver, may have toxic effects on the tissues of the host and cause necrosis and colliquation; as a result, a reaction is produced in the surrounding tissue.

A description of the lesion, after the mite has been detached, follows. The first case was examined two days, the second several days after detachment of the mite. Both show similar changes. The epithelium has disappeared and the papilla of the corium is surmounted by a bleb containing serous fluid, with fibrin and leukocytes. It has not yet become a true pustule. The changes in the corium are slight,

although there is infiltration by migrating cells and distension of the blood-vessels, the cellular exudate is composed of mononuclears. The epithelial cells surrounding the cyst are undergoing vesiculation. The boundary between corium and epidermis is more disarranged in the second, than in the first case examined. A comparison of these with the changes seen in a fresh akamushi-bite shows them to be quite alike, the only difference is that one is fresher than the other and there is nothing to show any progressive change. Bacteria are not found in these lesions and since the process has been followed throughout its course, we can say that the changes are due to the inflammatory reaction to the venom of akamushi. The changes in monkeys and rats do not differ from those in the human, excepting in degree.

B. Infectious Bite Lesion.

(Primary Lesion of the Disease).

It is impossible to tell whether one has been bitten by an infectious akamushi until the causative agent of tsutsugamushi has been discovered. In spite of the removal of the tissue surrounding the bite the disease may develop, but if the virus is still confined to this area it may be removed before it has diffused. This must be done very early to be successful. There was one case in which the tissue was excised twelve hours after the bite was inflicted, but the virus had already become diffused and the disease developed. Hayashi also had an experience with a case in which the tissue surrounding the bite was removed about a day after its infliction and the disease developed in six days. Thus it is seen that the virus diffuses to other parts very rapidly although the amount of tissue removed, the interval before removal, and the virulence of the virus must all be taken into consideration. When the virus is still confined to the site of inoculation the only way to demonstrate it is to study its effects in the removed specimen. There must be some histological difference in the reaction to the virus and that to the simple venom. To determine this, comparative examinations of both reactions must be made at various stages in their development.

Many patients reported at our station within thirty-six hours after having been bitten and the examination of their

tissues showed a greater reaction than was observed in the case of simple, non-infectious bites. Special consideration will be given to this later on.

A boy of seventeen, who had not been previously bitten by the akamushi, came to the station complaining of slight headache and lassitude. He had discovered a bite in the left arm-pit the day before. There was no swelling of the regional nodes. After removal of the surrounding tissue tsutsugamushi disease did not develop.

Examination of this tissue showed the bite at the center of the left axillary fossa; the skin around it was slightly elevated. Microscopically there was a depression of the epidermis and near its middle the palpal claws of the mite could be seen running obliquely into the epithelium. There was necrosis of the epithelial cells beneath the horny layer and the lesion was sharply demarcated from the surrounding tissue. There was a sucking organ still in situ, but I could not follow it throughout its entire course. A cyst lay between the altered epidermis and the corium; it was filled with polymorphonuclear leukocytes, mononuclear phagocytes and fibrin. At the outer margin was a part of the corium which stained red with Van Gieson or eosin, and was more or less linear in its arrangement. It contained many nuclear fragments. At the deeper margin of the cyst was a narrow layer of tissue, staining red with eosin, but of indistinct structure which was made up of fibrous bundles and fragmented nuclei. There was vesiculation of the nuclei in the epithelium, which was sharply separated from the altered portions. The epithelium was edematous and the intercellular bridges stood out distinctly. The above changes do not differ materially from those of the ordinary akamushi bite, but there are differences which we will now consider.

In the simple bite there is merely slight infiltration of leukocytes around the cyst, but in the infected bite this is very marked and the cyst is surrounded by a diffuse mass of migrated cells. This extends out to the capillaries and sweat glands. The cells are of two types: polymorphonuclears and mononuclear phagocytes. The latter have an ovoid or rounded nucleus which is poor in chromatin and possesses one or two distinct nucleoli. The cytoplasm is pale. These two varieties of cell mingle around the cyst, but the polymorphonuclears decrease in number as the distance from the cyst is increased and finally only mononuclears are seen.

Surrounding the cyst in a narrow compass there is irregularity of the corium and epidermis, masses of polymorphonuclears and vesicular cells lie beneath it, and the epithelium is invaded by leukocytes.

As we have already said, in this particular case the tissue surrounding the bite was removed and the disease did not develop; therefore it is difficult to decide whether the virus was completely removed with the tissue, or whether this was

merely a marked reaction to akamushi venom. We can arrive at a conclusion only through histological studies. The changes noted near the site of the bite do not differ very much from the simple akamushi bite lesion, but there are very much more extensive changes in the surrounding tissues than usual in that case. The large area of mononuclear phagocytes is quite characteristic and is supposedly in close relationship to the extent of dissemination of the virus of tsutsugamushi disease. There are many polymorphonuclears, but their distribution is quite limited as compared with the mononuclear cells. Whether their presence is due to secretions of the mite, or to bacterial contamination is doubtful, as it was impossible to make successful bacteriological examinations. The mononuclear cells are large lymphoid cells or macrolymphocytes. They are also found in the simple bites, but a marked increase in their number may indicate the infectious type.

We may, therefore, assume that this is a case of infectious bite, but the lesion would have to undergo further change to become a typical one. We were unable to examine these changes systematically in the varying stages of papule, vesicle, pustule and crusted lesion; but among the specimens we removed there were tissues which had not yet become completely altered and showed fresh changes in the surrounding tissue, in which we could form an idea of the intermediate changes. The well-developed bite lesion follows a different course, depending upon whether or not the disease be typical, or atypical tsutsugamushi.

a. Atypical Bite Lesion.

The tissue removed from three atypical cases (12, 31, and 47 years old respectively) was examined, the macroscopic findings being the same as in typical cases. There were flat brown crusts with reddish areolae, no infiltration or tenderness.

Histologically: There is no epidermis over the surface of the lesion; it shows a crust consisting of hyalinized, desiccated connective tissue and pus. In two cases there were underlying abscesses. The adjacent epithelium is vacuolated, the layer of corium about the abscess is very thin and necrotic and this, in turn, is surrounded by a zone of perivascular infil-

tration of lymphocytes, which also follow the sweat-glands and their ducts, and the nerve fibers. This infiltration extends to the deeper layers of the corium. The papillae around the abscess cavity and beneath the necrotic epidermis are edematous and contain serous and serofibrinous exudate; they are irregular in outline. The fluid may also be seen between the corium and epidermis, elevating the latter. The infiltrating cells are made up of large numbers of large mononuclears, large lymphoid cells, microlymphocytes and a few mast cells. The number of polymorphonuclears is increased near the abscess.

The abscess appears to be formed by an inflammation of the papillae in the upper layers of corium, which become softened and break down; suppuration is due to the presence of a large number of cocci and bacilli in every case, and that it may have very little connection with tsutsugamushi virus is indicated by the gradual decrease in number of polymorphonuclear leukocytes.

When these lesions are compared with those of the earlier stage of invasion, it is seen that there are fewer infiltrating cells in the latter and the necrosis has not extended as deeply as it does in the typical, fully-developed lesions.

There are bite lesions in atypical cases in which the picture is quite different from those just described; we had three such cases, two of second attack and one in which there were two bite lesions, one typical and one atypical. Histologically, all three cases showed epithelial necrosis and destruction of the superficial layers of the corium, exudative inflammation, cellular infiltration along the vessels, nerves, and sweat glands; but these changes were less pronounced than in the typical cases. The main difference was a marked increase in the number of clasmatocytes, which infiltrated the subcutaneous tissue deeply. Softening and granular necrosis followed.

b. Typical Bite Lesions.

In order to study the various stages of development in these lesions the following nine cases were examined; in seven the tissue was removed at biopsy, in two at necropsy.

Age	Sex	Days intervening between onset and removal of specimen
(Biopsies)		
14	F	3
30	M	4
25	F	5
14	M	6
70	M	8
34	M	9
17	M	about 19
(Necropsies)		
31	F	9
57	M	about 10

Histological Findings: Generally speaking, in wounds, removed a few days after onset, the epithelium, which is rich in cytoplasm, becomes necrotic and the lesion is covered solely by horny cells. Immediately beneath these there is a cyst with sero-fibrinous contents and necrotic corium, either adhering to the horny layer, or detached from it. Adjacent to this there is necrotic and softened epithelium. The base is formed by a thin layer of broken-up corium with fragments of epithelium and papillae. The serofibrinous exudate gradually becomes purulent and polymorphonuclear leukocytes appear at the periphery of the lesion. As the lesion ages, it dries out and becomes encrusted with a light-brown honey colored layer. The crust is dry, adheres firmly to the underlying tissue, and stains dark. The nuclei of the surface cells become contracted, but those lower down undergo karyorrhexis and become granular. Masses of bacteria may be found on the surface, or just beneath it. The intercellular substance becomes dark and stains deeply with hematoxylin.

Beneath the crust the connective tissue of the corium, especially near blood-vessels, nerve fibers and sweat-glands, is found to be infiltrated with mononuclear cells. Blood vessels become ensheathed in masses of cells; there may be a few lymphocytes and polymorphonuclears among them, but plasma cells do not appear until later. The infiltration penetrates deeply into the subcutaneous tissue, surrounding the coiled sweat ducts and glands, and invading the fatty tissue. These cells are large lymphocytes or lymphoid cells which show more or less activity, engulfing lymphocytes and red cells. They show mitotic figures. The endothelium of the distended capillaries shows very little change and the vessels are filled with lymphocytes and mononuclear cells.

The other change noted in the corium is its necrosis, which extends from the middle of the lesion into the subcutaneous tissue. Sometimes it is sharply divided from the surrounding, normal tissue, but it may not be. The connective tissue fibers of the necrotic area lose their staining properties more or less, the elastic fibers surviving longest, but eventually becoming destroyed. The infiltrating cells lose their nuclei and become granular, finally being transformed into homogeneous masses. This cellular and nuclear destruction is very marked in the normal tissue surrounding the necrotic area, but the more distant cells are unaffected. In and around the necrotic focus there is a fibrinous exudate which may also fill the distended capillaries. There may be slight capillary hemorrhages.

The necrotic core may reach the surface or extend along the hair-follicles or blood vessels with the cellular exudate. While the crust is still light brown, its margins may still be in a fresh condition, with a marked sero-fibrinous exudate into the papillae which become edematous and irregular. The blood vessels are distended and show slight hemorrhages. There may be superficial necrosis, which may penetrate the epithelium, which disappears after undergoing softening, or the cells may unite and form a cyst containing granular or fibrinous material. Sometimes the infiltration of lymphocytes and polymorphonuclears into the papillae becomes very pronounced, involving the overlying epithelium. When the latter becomes necrotic its cells break up into granules and stain deeply. As this proceeds, or when the crust has reached the dark stage, the exudate dries out and the necrotic epidermis and corium adhere together, forming a combined crust.

If the necrotic area sloughs, for one reason or another, an ulcer is formed and granulation tissue, rich in plasma cells, covers its base and walls. The lymphatic vessels of the corium, especially in the subcutaneous areolar tissue, are filled with the cells already enumerated and become distended. Occasionally giant cells may be seen in the necrotic portions of the epithelium.

The important changes in this type of lesion are: colliquation necrosis of the epithelium, sero-fibrinous exudative inflammation of the superficial corium, superfical and deep necrosis of the corium, and infiltration of the lesion by large

numbers of mononuclear cells. This differs from the atypical wound in that the necrosis does not reach the deeper tissues in its case.

Summary: The bites of akamushi do not invariably develop into a true primary lesions of tsutsugamushi disease; only a small proportion of them do. When the akamushi is non-infectious, local necrosis of the epithelium is produced, with a very slight amount of necrosis in the papillae of the corium, and a small, superficial cyst is formed which contains sero-fibrinous exudate. The reaction in the surrounding tissue is slight. This necrosis and exudative inflammation, as already stated, is caused by the toxic action of the venom of the akamushi, and is very slight in comparison with that following the sting of some parasites having venom glands. The appearance of polymorphonuclear cells is due in part to the presence of this toxin, but chiefly to that of bacteria.

If the attacking akamushi carry the virus of tsutsugamushi, this is introduced into the skin and the changes seen at the site of attack no longer constitute a simple or non-infectious wound. Within a day and a half the reaction produced in the surrounding tissue is very marked, the vacuolization of the epithelium and the infiltration of mononuclears in the corium are very pronounced.

We did not have preparations showing all the progressive stages leading to the final formation of a necrotic cavity, but it may safely be assumed from the examination of tissues from old wounds, that necrosis and softening are produced in the epithelium surrounding the site of attack and that the superficial layer of the corium is also involved, forming a vesicle that contains sero-fibrinous exudate. Tanaka attributed this vesicle to the softening of the epidermis alone, saying that it is comparable to the vesicle of smallpox, the only difference being that it is not multilocular. My findings agree with his. The necrosed epithelium takes up water, becomes swollen and, by losing their cell membranes, the epithelial cells fuse to form a cyst, but the formation of this vesicle also involves the superficial layers of the corium. Besides this there is also stagnation of the exudate between the epidermis and the papillae of the corium.

The colliquation necrosis of the epithelium is only mani-

fested as a vesicle, but it extends to the surrounding tissue. A sharp demarcation between the normal and the involved epithelium, where the nuclei have degenerated and the cytoplasm stains faintly, may be noted. Immediately beneath the epidermis there is exudative inflammation of the papillae of the superficial corium and sero-fibrinous exudation. This may extend into the necrotic epithelium and there may be, in addition, hemorrhages into the papillae, with necrosis.

The central vesicle may show inflammatory reactions because of the presence of bacteria in the hair follicles, or introduced from without, so that later it may become a pustule. At this stage the crust is light brown and is composed of a horny layer, dry and softened epithelium, and exudate. This agrees with what Tanaka has said, but it does not represent the composition of the entire crust, which changes with the course of the disease.

Macroscopically the primary lesion is not indurated, but microscopically one always finds a cellular infiltration. This is seen in the connective tissue surrounding the wound and is very marked along blood-vessels, nerves, sweat glands and ducts. It extends deeply into the subcutaneous tissue among these glands and the fatty tissue. The blood- and lymph-vessels are swollen. The infiltrate is composed largely of large lymphocytes, or lymphoid cells, mixed with a small number of microlymphocytes, with which polymorphonuclears may be intermingled. Some of the mononuclear cells show mitotic figures, indicating cellular division; they often exhibit phagocytic activity, taking up lymphocytes and red cells. The plasma cell appears at a later stage. The connective tissue cells are also increased in numbers. When the wound is opened it becomes an ulcer lined with granulation tissue, composed chiefly of plasma cells. There are no indications that mononuclears are transformed into plasma cells.

The other important changes are the necrosis of the subcutaneous tissue deep in the corium. This may be considered to be a coagulation necrosis, judging from the presence of fibrin. The boundary is not always sharp; at first connective tissue fibers may be poorly stained, but the elastic tissue fibers retain their staining capacity longer. Gradually this is lost by the superficial portions. The cells of the necrotic

area show various changes, then break up and finally become granular. These changes are seen beyond the limits of the necrotic cavity.

Necrosis of the deep layers of tissue follows along the masses of exudate around the blood vessels and hair follicles, often extending into the subcutaneous connective tissue. Hayashi thinks that this central necrosis is connected with the sucking organ of the akamushi, but we could find no relationship between the two; moreover we can not conceive how such a specific alteration could be brought about by the mere presence of the sucking tube.

If the crust be not removed, the incrustation extends to the necrotic cavity; if it be removed an ulcerated surface is exposed. Such changes are seen only in the typical primary lesions; the necrosis of atypical wounds either fails to penetrate the deeper parts, or it is slight. The superficial changes in both types, however, are identical.

A case which took an atypical course was found among the material collected from second attacks and in this the superficial changes are not marked. Instead of necrosis of the deep tissue, there is a funnel-like exudate composed of clasmatocytes, which extends to the subcutaneous areolar tissue. The clasmatocyte may appear more or less frequently in the typical case, but it is never as numerous as it is in this one. Here, clasmatocytes are also found in the superficies.

Examination of the tissue taken from a healed bite scar in a man with a second attack (age 29 years) shows some induration and while microscopic study shows no changes in the epidermis, there is a marked infiltration of round cells in the corium and subcutaneous tissue, these being chiefly microlymphocytes massed about the blood-vessels and among the connective tissue fibers. The endothelium of the blood vessels is more or less swollen, but not increased. There is no hemorrhage or necrosis. The pigment cells are increased in number. The probable reason why the picture is not typical in this case is that the man had more or less immunity from his first attack. The same reason may explain the atypical lesion in the man with two bite lesions.

The diseases which cause local skin necroses are plague,

carbuncle, and chancroid. In plague and carbuncle the chief components are pus cells; while in the chancroid the macroscopic picture is very similar to the primary lesion of tsutsugamushi disease, histologically, however, there are many pustules formed beneath the epidermis, with a marked infiltration of plasma cells and, in the presence of the Ducrey-Krefting bacillus the tissue becomes necrotic. In cases of tsutsugamushi disease the appearance of plasma cells occurs in the very late stages and there is no tissue necrosis as in chancroid. In the syphilitic chancre the infiltrate consists chiefly of plasma cells and the ulcer is definitely indurated. The tissue changes in glanders and Oriental boil are different. The skin changes seen in tsutsugamushi disease are, therefore, quite characteristic of that disease and clearly indicate specificity.

4. Pathology of the Lymphnodes.

Attention has been called to the fact that there is swelling not only of the regional, but of the systemic nodes in this disease and Tanaka and Hayashi have made detailed studies of this phenomenon. Tanaka divides the glandular swelling into two types: the primary bubo, in the regional nodes, and the secondary bubo in the systemic. He claims that they show different histological pictures. In the primary bubo karyorrhexis and coagulation necrosis are the chief processes, extending to the cortex and follicles along the blood vessels. In the secondary bubo necrosis may be only slight, or entirely absent. The endothelial cells are much increased, this increase being noted along the course of the vessels of both medullary and cortical portions and between the follicles. These cells are round or polygonal and mingled with lymphocytes. They show vacuolization and contraction, part of the former phenomenon being due to phagocytic activity. He observed cells filled with purple-stained granules of varying sizes. He explains that, while the causative agent enters the lymph sinus and acts upon the lymphocytes in a concentrated form, causing necrosis in the case of the primary bubo; in that of the secondary bubo, the virus is diluted with blood and stimulates the vascular and sinus endothelium as a consequence, without causing necrosis.

Hayashi noted hyperplasia and necrosis of lymphnodes, the regional nodes containing large as well as small foci of necrosis. The small foci are also found in other organs, decreasing in number as the distance from the primary lesion is increased. He stresses the importance of focal necrosis in this disease. The important elements in this lymphoid hyperplasia are large mononuclears and endothelial cells; there is no marked increase in lymphocytes, and he claims to have observed his "granular giant cells" in these nodes.

For convenience in description we have divided the lesions in lymphnodes into two classes: primary (regional) and secondary (systemic) lymphadenitis. We removed nine specimens from patients, at biopsy; eight during the first, and one from a case of second attack.

	Time of removal after onset	Number of cases. Age of patient
Biopsies		
	First day	1 (M, second attack, 29)
	Second day	1 (M, mild attack, 19)
	Third day	1 (F, died, 33)
	Fourth day	1 (M, died, 30)
	Fifth day	1 (M, severe, 34)
		(F, mild, 25)
	Sixth day	1 (M, mild, 14)
	Eighth day	1 (M, mild, 15)
Necropsies		
	About nine days	2 (F, 32 and 33)
	About ten days	1 (M, 42)
	About three weeks	1 (F, 42)
	Two weeks in convalescence	1 (M, 48)

a. Primary Lymphadenitis.

The lymphnodes nearest the bite are swollen to the size of a pea, or to that of a pigeon's egg; the capsule becomes tense but there is no adhesion to the surrounding tissues. The cut surface shows the medulla to be red, or grayish-red and there may be grayish-yellow necrotic patches or cavities beneath the capsule. Histologically there is an increase in the number of vesicular cells which are found chiefly opposite the hilus, in the more medullary portion of the node. The follicles are contracted and the marginal and interfollicular sinuses are occluded. These cells are chiefly large ones with a lightly stained cytoplasm and a vesicular nucleus. Mixed with these, or lying discrete, lymphocytes and a few fibroblasts may be found. The endothelium of the capillary vessels is more or less swollen.

The entire medulla of the organ is not involved; surrounding the affected areas are distended sinuses filled with vesicular cells, lymphocytes, plasma, and red cells. There is a strikingly large number of plasma cells in the follicles; these are of the Marschalko, "wheel-nucleus" type, usually showing one nucleus, but occasionally several. Mitotic figures may be present. As these cells are found where there are many lymphocytes, their production from the latter is indicated. Their cytoplasm becomes transparent, or vacuolated, the nucleus vesicular and they can not be distinguished from the vesicular cells; after they break up they remain only as masses of cytoplasm. They are also found in small numbers at the center of the areas of medullary inflammation and may also enter the marginal and follicular sinuses as already mentioned. Sometimes giant cells with lobulated nuclei are found in the sinuses.

The vesicular cells are usually mononuclear, but they may have two or three nuclei and a few of them may show mitotic figures. Their cytoplasm stains poorly, may contain vacuoles, and they show phagocytic activity, taking up lymphocytes, plasma cells, erythrocytes, or bits of nuclear fragments. This activity is most marked in the sinuses. As a rule these cells contain but few fat droplets, even when they are outside of the necrotic areas, but there are instances where they may be filled with them. The parent cells of this type are chiefly reticulo-endothelial, but a few of them arise from plasma cells and lymphocytes. Mitotic figures indicate that they may multiply in their vesicular form. The cellular infiltration, consisting of lymphocytes, plasma cells, and vesicular cells, involves the capsule and makes it irregular, and may also involve the surrounding fat; but as the perilymphatic inflammatory reaction is very slight, the nodes do not adhere to the surrounding tissue.

Besides the increase in cellular elements, there is always necrosis in the form of a zone beneath the capsule, or involving it. The margin of this zone is irregular and serrated, but there is always a sharp line of demarcation between it and the normal tissue. Around the larger areas there may be a number of smaller necroses which are found in the cortex and follicles. Near the edge of the necroses there are still

formed cells, but near the center they become granular and finally disintegrate. Karyorrhexis is seen everywhere; surrounding the necrotic focus there is a lighter zone composed only of vesicular cells with disintegrated nuclei and containing many fat droplets. Here there are no plasma cells. Even in the smaller foci of necrosis karyorrhexis is very marked. There is fibrin in these areas, as Hayashi has already pointed out, and this forms a reticulum in the swollen vessels and in their walls, as well as between the necrotic cells, being always found where there is necrosis. At times it may be seen in normal blood vessels and the tissue surrounding the necrotic foci. The lymphoid reticulum becomes hypertrophic and is, at first, well preserved; but later some of the finer fibrils are destroyed and only the coarser fibers remain. The degree of destruction of these fibers is more marked than in the simple necrosis. In a few instances there is an increase in the number of endothelial cells in the marginal sinuses and a marked fibrinous exudate. The endothelium soon becomes necrotic.

Occasionally hemorrhages are marked in the necrotic foci and there may be hemorrhages into sinuses in the medulla. They are due to congestion of the capillaries that follows the destructive occlusion of the veins by vesicular cells, rather than to the toxic effect of the virus.

The foregoing marked changes, increase of cells and necrosis, are seen in those regions where the action of the virus has been markedly toxic. They are most marked at the hilus, where it first enters the nodes from the area of the bite; but necrosis is not specific, as Tanaka seemed to think. Even in some of the fatal or very severe cases there was no necrosis beneath the capsule. In four out of our nine cases (one each of mild, moderate, severe, and fatal character) there was no necrosis; but there was isolated cellular necrosis, or cytorrhexis, in every instance. The extensiveness of necrosis does not necessarily depend upon propinquity to the bite; the lymphnodes directly beneath the lesion may show no necrosis while, on the other hand, it may be marked in some of the distant nodes. Therefore it is produced only when the virus acts strongly upon the lymphnodes. The change, which is truly characteristic and specific in this dis-

ease, is the increase in the number of cells in the nodes, chiefly in that of the vesicular and plasma cells. When the toxin is very concentrated the regional nodes are involved one after the other in this cellular increase and necrosis; but the changes produced gradually become less marked as the peripheral nodes become involved. Distant lymphnodes may, however, show necrosis.

The late course of the primary lymphadenitis was examined in the case of a patient who died in the second week of convalescence, and it was seen that the lymphoid tissue had recovered almost to normal, excepting that the sinuses were much distended with phagocytic cells within which were erythrocytes and a few lymphocytes. Cells rich in cytoplasm were scattered among the follicles. There was no increase in the trabeculae but the elastic fibers around the vessels were increased. The capsule showed an inner layer of interlacing elastic fibers, a vascular layer and a reticular, the last hypertrophied. No plasma cells or droplets of phagocytosed fat were observed.

b. Secondary Lymphadenitis.

The virus of tsutsugamushi diseases enters the blood through the lymphatics and acts upon the entire body, producing generalized swelling of the lymphnodes, all of which become enlarged whether superficial or deep. Thus we find the mesenteric and other abdominal groups, with the bronchial nodes, all enlarged. There is not as much induration as in the case of the regional nodes; the cut surface is grayish red in the medullary portion.

Histologically, the follicles and follicular cords contain an increased number of vesicular and plasma cells, the latter arising from lymphocytes, as already noted in the regional nodes. The increase in the number of vesicular cells in the cortex is very marked, where they form a paler zone, but they are only found in small numbers in the medulla. The pale zone may be extensive, or may alternate with normal lymphoid tissue, or it may be found quite isolated. The marginal and interfollicular sinuses become swollen and contain vesicular and plasma cells, lymphocytes and erythrocytes, the last being found lying between the cortical cells. The

vesicular cells are often vacuolated, may show phagocytic activity, taking up lymphocytes, plasma cells and red cells, and they may show mitoses, as in the case of plasma cells. Dilatation of the blood vessels is generally marked. There may be slight cellular infiltration into the capsule and outlying fatty tissue.

Necrosis may be observed in the visceral lymphnodes, such as the mesenteric, not only small focal necroses of the marginal or interfollicular regions, but also extensive areas. In such foci karyorrhexis is marked.

During the stage of convalescence the lymphoid tissue of the visceral nodes has almost regained its normal appearance; the sinuses are distended with cells rich in cytoplasm, polymorphonuclears, lymphocytes, plasma cells and erythrocytes. The endothelial cells may phagocytose lymphocytes and red cells. There may be an increase in the connective tissue of the follicles, but the reticulum is not increased. In a few places an increase of the adventitia and media of the larger vessels may be noted. Plasma cells may be found in small numbers, in the follicles, and are somewhat altered in appearance.

A comparison of the primary and secondary lymphadenitis shows that it is of the same character, differing only in respect to the degree of alteration. Cells are increased in number and there is necrosis, the former being found in the medulla and sinuses, with vesicular cells playing the most important part. Owing to their presence, the node becomes grossly paler. The distribution of these changes differs in the two types of lymphadenitis: in the primary, those nodes receiving the virus directly from the bitten area are most affected; in the secondary type, all the nodes are equally affected, as the virus is carried equally to all of them through the blood stream. In these nodes the cortical portion is more deeply affected than the medullary, and the sinuses suffer earlier than do the follicles and follicular cords, resulting in a picture of cortical inflammation. The cellular infiltration of the parenchyma extends through the capsule to the surrounding tissues. This is invariably seen in the primary lymphadenitis, but may be seen seldom, or not at all in the secondary. Necrosis is frequent in the primary lymphadeni-

tis, but it is not specific for tsutsugamushi disease, as claimed by Hayashi and Tanaka. Its extent varies. Beneath, or in the capsule, there may be found focal necroses of the cortical portion of the nodes, particularly of the follicles. It is of the coagulative type, as claimed by Tanaka, as it shows much fibrin precipitation; it is often found in cell masses, but it may be observed in marginal sinuses where there is no particular increase in the number of cells. With time, necrotic areas may become granular and present the appearance of caseation, with many fat droplets present. There is an accumulation of fat in the cells surrounding the necrotic areas, as well as in the intercellular spaces; it may also appear in the muscular coats of blood vessels, or in their intima. In the secondary lymphadenitis necrosis is rather exceptional, but sometimes the visceral nodes may exhibit a few foci.

Tenderness of the regional nodes, occasionally met with in the systemic, is not due to necrosis but to the cellular increase; however, it is difficult to say whether this tenderness is caused by the unequal degree of infiltration, by distension of the capsule, or by infiltration of the latter. It may be present when infiltration is not marked. The changes in the nerve filaments in these cases have not been determined.

Similar pathological alteration in the lymphnodes may be produced by typhoid, trypanosomiasis, and by syphilitic bubo. In typhoid the lymphnodes of the mesentery become swollen and necrotic, whether or not they are tender is not known. No general enlargement of lymphnodes is seen in this disease, so that there is no secondary lymphadenitis. Histologically there is a marked increase in vesicular cells, mostly in the sinuses, so that the follicles and follicular cords become much compressed. According to Schmidt, these vesicular cells are found among the smaller cells of the follicular parenchyma and the enlarged nodes, therefore, are composed of a mixture of the two; there is however no marked infiltration by plasma cells, as is the case in tsutsugamushi disease and there is more phagocytic activity on the part of the vesicular cells in typhoid than in that disease. The necrosis, in typhoid, occurs in both large and small cells and the capsule may be involved in that disease as well as in tsutsugamushi disease. Schmidt has noted the presence of the large cells in the sinuses of nodes

showing no macroscopic alterations, such as the cervical, inguinal and axillary groups, but this is rare in typhoid. Tsutsugamushi disease differs from typhoid in that the regional nodes alone are involved in the latter, whereas all the nodes suffer in the former.

In trypanosomiasis, the lymphadenitis denotes disease of long standing; the cervical, inguinal and axillary nodes are involved and may attain the size of peas, or even of pigeon's eggs. They may form nodular packets; there is slight, or no tenderness; there are many plasma cells and lymphocytes in the picture, but no tendency to softening or suppuration. A comparison of the lymphadenitis of this disease, with that of tsutsugamushi disease, shows that one is chronic, the other acute; the former has no separable primary and secondary types of lymphadenitis, as has the latter. The presence of plasma cells is common to both, but trypanosomiasis shows no tendency for the lymphnodes to break down.

Syphilis closely resembles tsutsugamushi disease in respect to its lymphoid pathology; upon contracting syphilis, the regional lymphnodes become indurated after three weeks or so; they swell at the middle or end of another two weeks, but show no tenderness. There is no perilymphadenitis and adhesions. When the treponema enters the blood, it involves the entire body and as a sign of this all the lymphnodes become swollen about six weeks after infection. Therefore that disease runs a course quite similar to that of tsutsugamushi disease, so far as the lymphnodes are concerned, but it is a very chronic one and, moreover, the regional nodes are not tender in syphilis, whereas they are very much so in tsutsugamushi disease.

According to Virchow and Orth there is a cellular increase in the syphilitic lymphnode. Cells resembling lymphocytes and epithelial cells fill the follicles and sinuses, the latter containing most of the epitheloid cells, which gives an effect of homogeneity. Not only does the parenchyma become hypertrophied, but there is an overgrowth of the capsule, sheath, blood-vessels and reticular tissue. Finally there is fatty infiltration of the parenchyma and caseous cavities, which are scattered through areas of normal tissue result. Accord-

ing to Jesionek, these changes occur both in primary and secondary lymphnode lesions and there is no difference between the two.

The increase in large cells closely resembles that seen in tsutsugamushi disease, but the degenerative changes in the plasma cells and the fatty infiltration are not described in the case of syphilis. In tsutsugamushi disease this is a process of coagulation necrosis. The reason why there is no great increase in the connective tissue in tsutsugamushi disease is that this process is very acute, and the pathological changes in the two disease are therefore different.

After what has just been said, we may safely assume that the pathological changes in the lymphoid tissue in tsutsugamushi disease are peculiar to it and not similar to those seen in the case of other types of infection.

5. Pathology of the Accessory Lymphoid Tissue.

Macroscopically, there is no hypertrophy of the tonsils or the lymph follicles of the digestive tract; microscopically one notes an increase in the number of plasma cells. This is very marked in the tonsils, which also show the vesicular phagocytes.

6. Pathology of the Eruption.

It is well known that the rash has a very close pathological relationship to the etiological agent in most exanthematous diseases; therefore it should be studied from the histological and etiological, as well as from the clinical standpoint. Comparative study of the rashes of the exanthemata of unknown etiology is very important. It is much better to obtain the material for histological study from living, than from dead subjects; but as it is not always easy to get it from the living, we must content ourselves with an incomplete series of specimens.

For the study of the rash we obtained material from six living patients and one cadaver, representing stages three days (1), four days (2), nine days (1 cadaver), seventeen (1), and twenty-one (1). In the last the eruption had faded until only dark spots remained. In the removal of these specimens local anesthesia with novocain was employed.

Histological examination shows that there is at first a

surrounding of one to three arteries in the lower layers of the corium, or an accumulation at one side of them, by cellular masses. These do not form nodules, but rather perivascular exudates extending as far as the vascular rate in the upper layer of the corium, where they surround the small arteries beneath the papillae. Between the connective tissue fibers of the upper layer of the corium and the papillae one finds patches of anastomosing of branching cells, most prominent at the center of the papillae. The sweat ducts are surrounded by a narrow strip of cells in the vascular zone around the duct, but the hair follicles are not surrounded. The papillary blood vessels are dilated and, at the height of the eruption, the veins also become congested.

The cells surrounding the blood vessels are of two main types: mononuclears, and lymphocytes rich in chromatin and poor in cytoplasm. There are no polymorphonuclears present. The mononuclear cells are divisible into three subtypes: a. Cells with deeply-stained, rounded, irregular, or dumb-bell shaped nuclei and a varying amount of cytoplasm. b. Cells with rounded, elliptical or ovoid nuclei which stain lightly, and a good deal of cytoplasm. c. Cells with a stellate or branching outline with nuclei rich in chromatin and rather poor in cytoplasm. Various transitions between the first and third type are observed, but only a few of the second type are found. As the disease progresses, there is an increase in the number of the second type and when the eruption is fading it becomes the most numerous variety, when lymphocytes may appear in company with it. The cells found among the connective tissue fibers are about the same as the foregoing varieties; but there are more of the third, than of the other types. This is especially true in the early stages of the disease. These cells are not only found in the connective tissue, but they may also wander among the epithelial cells causing the epidermis, and the papillae especially, to become broken-up and thinned-out. This is most marked at the center of the papule. Mast cells are not increased, but a few may appear in the lesions during the fastigial or defervescent stages. Plasma cells may be seen around blood vessels and in the connective tissue, but are more numerous around the former. No transitions between other cells and

plasma cells are noted, but these show many mitotic figures, which indicates rapid proliferation.

From the outset the mononuclear cells show vacuolization and, as the disease progresses, they increase in number. In the defervescent period the nuclei show fragmentation, shrinkage and disintegration. Vacuolization and reticulation of the cytoplasm are distinct. These changes may be noted in single instances or in groups of cells. The phagocytic activity of these cells is not great during the early or fastigial stages, only a few of them taking up lymphocytes, but during defervescence it becomes very marked, many nuclear fragments being phagocytosed.

In the early stages of the disease the cellular masses around blood vessels are compact and intercellular fibrils few in number, but by hypertrophy and proliferation, they finally become transformed into connective tissue fibers during defervescence. At this time the elastic fibers become destroyed. The outer wall of the arteries, surrounded by these cellular masses, is infiltrated by the cells, but the media is intact and the endothelium often swollen. This change in the endothelium is sometimes very marked in the case of arterioles and capillaries. At times the adventitia of capillaries becomes hypertrophic. In the defervescent stage, the lumen may become filled with fibrin. The veins may be dilated during the fastigium, but their walls are unchanged. There are no changes in the lymphatic vessels.

The bundles of nerve fibers, which are involved in cellular masses, are not infiltrated, but in the fastigium or during defervescence, the fibrils of small bundles may atrophy and disappear.

Changes in the papillae are mainly due to cellular infiltration of the connective tissue, but there are no changes in the connective tissue fibers; consequently the margin between epidermis and papilla seldom shows irregularity. The other change noted is necrosis; in one specimen two necroses were found at the tip of papillary bodies. This is of the coagulative type in its early stage, a space forming between the epithelium and the papilla and the former undergoing necrosis. In no stage was there any change in the blood-vessels, nor were there hemorrhagic foci.

As to the epithelial lesions: there are compression and destruction of the epithelial cells due to infiltration by wandering cells, and occasionally there may be vacuolization and necrosis of the epithelium overlying the necrotic papillae. Vesicles of variable size are formed by the liquefactive necrosis in the granular layer in which yellowish-brown pigment granules and wandering cells may be found. The horny layer may be preserved, together with one or two rows of cells in the granular layer or it may be completely destroyed. In defervescence there is increased cornification and hypertrophy of the horny layer at the center of the lesion, but the cells retain their nuclei. It presents the picture of parakeratosis. One may find many nuclei and their fragments, and yellowish-brown pigment in some portions of the horny layer; beneath such areas the germinal layer is very thin and the demarcation between it and the connective tissue vague. Pigment cells may be found in such areas.

The vesicular eruption, which is occasionally observed, is seen to be situated in the epithelial layer. The character of such vesicles may be much like that of miliaria crystallina, indicating some communication with the sweat ducts. There is no cellular infiltration around or in the cysts, the contents of which are granular and stain lightly with eosin.

The chief histological changes of the eruption, then, are the increase of mononuclear cells around the blood vessels, (especially arterioles at the point of bifurcation) and in the connective tissue, with occasional necrosis of the papillae.

The infiltrating cells are mononuclears which may be classified as microlymphocytes, macrolymphocytes and wandering cells. The latter are not brought in by the blood stream, but originate and multiply in situ, arising from the adventitia of blood vessels, or from their fibroblasts. In the connective tissue they arise from cells found among the fibroblasts. That these are wandering cells may be demonstrated by the fact that they are found more abundantly in the connective tissue near the cellular groups about blood vessels and between the cells of the epithelial layers. They show regressive changes in the defervescent period and the intercellular material becomes hypertrophic and forms small cicatrices. An interesting point to note is the appearance of many

plasma cells in the cellular aggregations during the later stages of the process.

Despite the fact that there are cellular masses around the blood vessels, these are not involved; there is no change in their intima, other than occasional swelling of its endothelium. Changes in the capillaries are confined to the swelling of their endothelium and only in the defervescent period are they filled with fibrin, a feature that has some bearing upon the atrophic changes seen at that time. The necrosis of the tip of the papilla and of the epithelium covering it is of the colliquation type and has no connection with the intracapillary changes. This may be a result of the action of the virus. With the increase in the cellular exudate the papillary blood vessels become dilated and there is local increase in cells around them, particularly around arterioles. Thus the areas of cellular proliferation are gradually extended and reach a maximum in the fastigium.

A few words may be said as to how far these histological findings coincide with the clinical phenomena. The eruption of tsutsugamushi disease is papular and pale red, on pressure it may fade to brownish. This is well accounted for histologically; the elevation of the papule is due to the distention of the papillae beneath the lesion and the perivascular cellular increase in the connective tissue, although there is no irregularity or swelling of the connective tissue itself; the color of the eruption is due to local congestion and, since it is just simple congestion, it may fade on pressure; as the tissue is elastic, the lesion flattens on pressure; the deepening in its color, as it ages, is due to increased congestion and, as there are no hemorrhages, even at this stage, it may fade out on pressure. The reason why the color can not be expressed in the later stages is because the epithelial pigments are increased and not because blood pigment has been produced by hemorrhage. In our specimens taken during the defervescent stage, there was an increase in melanin and a parakeratosis; but as there were macroscopically visible brown spots at the site of the eruption in these cases, we do not know whether the same explanation applies to those lesions which disappear and leave no trace. The infiltration of the epithelium by wandering cells breaks up the papillae, possibly stimulating

an increase in melanin and at the same time inducing parakeratosis, but the degree of such changes differs according to the type of eruption. A crust forms over the papule when there is marked colliquation necrosis of the epithelium. When this dries out the crust is formed, which includes the horny layer of the epithelium.

Nerve bundles are often buried in masses of exudate and their fibers show atrophic changes, but there is no pain nor itching; likewise the bite lesion is accompanied only by very slight prickling sensations. This may be due to the fact that the toxin of the virus of this disease may paralyze the sensory nerve endings.

That the formation of small vesicles in the eruption is due to a fusion of multilocular cysts caused by colliquation necrosis in the epithelium is demonstrated by microscopic examination; but, on the other hand, such cysts are closely associated with the ducts of sweat glands and, as there is profuse perspiration in the early stages of the disease, increase in sweat-secretion may facilitate cyst formation. These cysts are, however, much smaller than true miliaria crystallina and they appear at an earlier stage; later miliaria may be combined with the eruption

Thus we see that the histological findings closely correspond with the clinical; we shall now consider whether or not similar phenomena are seen in the eruptions of other diseases.

In typhoid the eruption is red, slightly elevated, and its lesions round; its color fades on pressure in all stages. (Curschmann (22-23)). It closely resembles the eruption of tsutsugamushi disease clinically, but shows a very different histological picture. Fraenkel (27-29) removed an area of typhoid roseola aseptically, placed it in a tube of bouillon and incubated it for eighteen hours. He then examined the tissue and found that the typhoid bacilli had multiplied and assumed branching forms, being found in the lymphatic channels of the reticular portion of the skin and in the papillae. These were swollen and the boundary between epidermis and corium was irregular. He also noticed necrotic areas in the papillae and epithelium and at times many lymph vessels were filled with coagulated lymph.

In measles the eruptive lesion is round, light or deep red, and very slightly elevated. At times it may be acuminate. Each papule is developed independently of its neighbor and there is no fusion of the lesions, (Immermann (55)). Reports of histological examinations have been made by Cartrain, Unna (178), and Feldberg and detailed studies by Ewing (26), and Mallory and Medlar (96). Recently Abramow (1) has made a careful study of the eruption. Blake and Trask (15) and the writer (62) have reported on studies of the rash in experimental measles. There are changes in the epithelium and corium; in the latter there is edema, swelling, and cellular infiltration around the capillaries. The infiltrating cells are mainly epithelioid together with lymphocytes, fibroblasts and a small number of neutrophil and eosinophil polymorphonuclears. The epithelioid cells are the "endothelial leukocytes" of Mallory and Medlar, and of Blake and Trask. They may be regarded as large lymphoid cells, or as leukocytoid wandering cells. As to the changes in the epithelium, there is vesicular swelling of the granular layer and necrosis. Small vesicles are formed by lysis and, uniting, these form cysts. There is an infiltration by wandering cells extending out from the corium, but as the lesion is due to changes in the epithelium, it is not caused by the presence of these cells. The change in the epidermis is a parakeratosis caused by the toxin of measles.

The syphilitic eruption also takes the roseolar form, especially when it is fresh. There is a heavy cellular infiltration of the papillae and subpapillary vascular rate. When the eruption is fresh the cells are made up chiefly of leukocytes, but after a few days many plasma cells appear. Polynuclear giant cells may be found at the height of the disease or in the defervescent stage (Ehrmann (25)).

In typhus and Rocky Mountain spotted fever there are also eruptions. The etiology of neither of these has as yet been settled, but their biological characteristics resemble those of tsutsugamushi disease very closely. The eruption of typhus is macular, in contrast to the papular lesions of tsutsugamushi disease; it may be made to fade under pressure at first, but later becomes hemorrhagic and will not pale under the fingertip. Histological studies of the typhus lesion have been made

by Fraenkel (27-29) who found nodular cellular proliferations around the arterioles of the skin and localized necroses of the arteries. These changes have been confirmed by many investigators (Ceelen (19), Benda (13), Bauer (12), Gruber (35), Kyrle and Morawitz (92)) and have further been noted in the vessels of the cerebral cortex and various viscera. The necrosis of the vessels begins as a small exfoliation of cells from the endothelium and then the entire wall becomes involved and thrombosis results. The cellular exudation may be regarded either as proliferation or immigration; it is found as nodules of cells in the proximity of vessels, chiefly large lymphoid forms rich in cytoplasm and a few polymorphonuclears. The former come either from the adventitial cells or the perivascular connective tissue.

The eruption in Rocky Mountain spotted fever is at first pale red and fades on pressure, but later it becomes hemorrhagic and deep, or purplish red, the lesions increasing in size. It disappears with the fall of temperature, but petechiae persist as pigmented spots. In very severe cases there are skin necroses. Wilson and Chowning (181) reported that the capillaries are distended and filled with blood containing many leukocytes, with hemorrhages into the neighboring tissue, and a deposition of blood pigments in areas of older hemorrhagic eruption. There are a few phagocytic cells taking up erythrocytes. Wolbach (184) has described occasional lesions in the large vessels beneath the skin, in the form of small areas of endothelial proliferation and polymorphonuclears in the media. The most marked changes are in the capillaries about the sweat glands and papillae, their lumina are packed with mononuclears and the vessels are surrounded by masses of mononuclears (both ameboid and phagocytic), with occasional polymorphonuclears, mast-cells, or lymphoid cells. There are also extensive degenerative changes in the epithelium of the coil glands. A few nerve fibers show penetration by ameboid wandering cells. There are scattered areas of small hemorrhages and the capillaries may become plugged with fibrin.

A review of the studies on the eruptions of the foregoing diseases shows that the rash of measles is the only one closely resembling that of tsutsugamushi disease. The eruption of

typhoid fever resembles it in its swelling, increase of cells in the papillae and its absence of vascular necrosis and hemorrhage. But the distribution of the cellular exudate around the arteries is different. This is very marked in typhoid, where it takes on a nodular form and contains polymorphonuclears. The vascular necrosis and hemorrhage in the eruption of typhus are not seen in that of tsutsugamushi disease, the fact that the latter has no hemorrhages is a point clearly differentiating it from Rocky Mountain spotted fever. The syphilitic eruption of the secondary period is rich in plasma cells which distinguishes it from that of tsutsugamushi disease. Comparison with the rash of measles shows that this has an increase of mononuclear cells in the bifurcations of the arterioles of the corium and occasional necrosis of the papillae, but there are no necroses nor hemorrhages from the blood vessels. Atrophy and disintegration of epithelial cells, due to their infiltration by wandering cells, vesicular changes and parakeratosis are all noted as lesions of the epithelium. These characteristics resemble those of tsutsugamushi disease very closely. Thus we may consider the eruption in tsutsugamushi disease unique, if we except measles.

An important point that should be noted in connection with the rash of tsutsugamushi disease is that the changes seen at the site of eruptive lesions are identical with those seen at the site of the insect bite. As already described, there is cellular proliferation surrounding the vessels in the bite lesion. These cells may be considered to be large lymphoid cells or leukocytoid wandering cells, but they have arisen in situ. In the area where there is cellular infiltration many plasma cells are to be found later. That necrosis is often noted in the eruption is a point in common with the bite lesion. Thus we see that the skin lesions are essentially the same, whether they be noted in the lesions of the rash or of the bite and that they are caused by the stimulating action of the virus or its toxin.

7. Visceral Lesions.

A. *Spleen:* According to gross and microscopic descriptions by Tanaka and Hayashi, the spleen is swollen and congested and its parenchyma softened. Microscopically they

noted many red cells and giant cells in the pulp and that the latter was hypertrophic, exhibiting mitoses in its cells which were ameboid and wandering. Hayashi described atrophy and decrease in the number of follicles and small foci of fragmented nuclei.

We examined six cases which ran typical courses and one in which the patient died during convalescence.

Case	Sex	Age	Days after onset	Weight of spleen	Size of spleen
1	M	57	about 10	185	14.5 x 9.5 x 3.5
2	M	30	19	355	17.0 x 11.0 x 4.0
3	F	33	9	238	14.0 x 9.0 x 4.0
4	F	32	9	165	13.5 x 9.0 x 3.5
5	F	42	about 3 weeks	140	14.5 x 7.0 x 2.0
6	M	42	11	120	10.0 x 7.5 x 4.0
7	M	48	2nd week in convalescence	50	8.0 x 6.5 x 2.5

Macroscopic Findings: The tsutsugamushi spleen resembles that of typhus; its capsule is tense or may be slightly wrinkled, it is somewhat hard, the cut surface is dark to chocolate red in color, the pulp granular and swollen, and sometimes a little is removable on the knife-edge, though it may not be. The trabeculae are still distinguishable, but the follicles are indistinct.

Microscopic Findings: The follicles are contracted and often disappear, the pulp is congested and irregular and there is an increase in the number of pulp cells. Plasma cells appear to some extent. Around the margins of the pulp spaces there is some indication of the transformation of lymphocytes into plasma cells. The sinuses are filled with erythrocytes, and endothelial cells may be observed becoming detached and forming giant cells. These may be found in the pulp or in the venous sinuses; their nuclei are irregular and difficult to identify. These endothelial cells are phagocytic. The cells phagocytosed by these syncytia are chiefly erythrocytes, but plasma cells and polymorphonuclears, together with fragments of other cells may be found in their cytoplasm. They vary in number in different specimens and it is curious to note that they phagocytose cells which have already taken up red cells. Mitoses are seen in the pulp cells and in plasma cells. Some of the cells appearing in the pulp may be degenerated and the nuclei of the wandering cells may become fragmented.

Dark brown, granular pigment may be seen in the pulp, but it does not give the iron reaction.

Here there are many minute necrotic areas with indistinct outlines, within which are nuclear fragments and chromatin granules. The Bielschowsky method demonstrates that the reticular fibers are somewhat thinned-out, but they are well preserved and their meshes equal and wide. No fibrin is demonstrable by the Weigert method in the necrotic foci. The sinuses are dilated but their walls are unchanged. Fat is not usually increased, but fat droplets may be seen in the giant cells and pulp cells.

The specimen removed from the body of the patient who died in the convalescent period shows a thickened, tough capsule, brownish-red cut surface, firm pulp, and distinct trabeculae, although the follicles are scarcely recognisable. Microscopically the capsule is found to be thickened, the trabeculae increased in size, and the follicles markedly shrunken. There is a small amount of hyalin substance around the follicular arteries, the cellular elements of the pulp are greatly decreased in number and only a few plasma cells are found. Many pigment granules, giving the iron reaction, are found in the pulp and also (in smaller numbers) within detached endothelial cells. The endothelial cells of the sinuses may become cylindrical, presenting the appearance of epithelial ducts. The reticulum fibers of the sinus walls and of the pulp are thickened. Many large cells containing fat droplets are found in the pulp.

B. *Bone Marrow:* No one has reported concerning the bone marrow in this disease. We obtained specimens of bone marrow from six necropsies, one of which was the patient who died during convalescence, the material being obtained from the femur.

The specimens consisted of red marrow in the upper, white marrow in the lower half. Histologically it is rich in cellular elements and the blood vessels are dilated. Among the increased elements are myeloblasts, myelocytes, and lymphocytes; sometimes the last named form nodules. There is no increase in hematopoiesis and no decrease in the number of negakaryocytes. Plasma cells are increased in numbers and are found singly, or grouped; a transformation of lympho-

cytes into these cells is again not perfectly evident, but there is some indication that detached adventitial cells may become plasma cells. Besides these there are large cells already noted in the spleen and lymphnodes; their nuclei are elliptical or indented and they contain lymphocytes, plasma cells and erythrocytes, besides fragments of all these. They are pretty constantly found in all the cases we have examined, and may occur within blood vessels.

Besides this cellular hypertrophy, atrophic changes are noted; very small necroses were seen in one case, but cellular necrosis was found to a greater or lesser extent in all, the cells showing various nuclear changes or entirely losing their nuclei, their cytoplasm alone remaining as a granular mass. The material taken during convalescence shows red marrow at the upper half. Only a few regenerative cells are seen. A considerable number of phagocytic cells, containing lymphocytes and erythrocytes, are found in the capillaries and parenchyma. The cells become vacuolated and then degenerate into albuminoid masses. Brownish pigment, with a positive iron reaction, is found in rather large amounts. A few megakaryocytes are present.

C. *Liver:* According to Tanaka, the liver is not swollen and its parenchyma shows no particular changes. Hayashi noted that the lobules became indistinct and the arrangement of their cells irregular, calling this "dissociation."

Macroscopic Findings: Material was obtained from the seven cases already noted.

Case	Weight of liver	Size
1	1400	20 x 16 x 9 cm.
2	1470	26 x 17 x 8
3	1460	25 x 15 x 8.5
4	1070	24 x 12.5 x 8.5
5	1885	27 x 14 x 6.5
6	1380	28.5 x 15 x 8
7	900	25 x 11 x 6.7

Thus, except in one instance, the liver was swollen during defervescence. The capsule is tense, smooth and the liver border is blunt. The organ is slightly soft, its cut surface drips blood, its parenchyma is more or less elevated above the plane of the section, is swollen and has a dirty yellowish-brown color. The lobules may or may not be distinct.

Microscopic Findings: In the majority of the cases the liver cells are irregularly arranged at the centers of the lobules and are dissociated. The capillaries are congested. Minute necrotic areas are found in the lobules and cellular necrosis is noted in the cellular masses. The former were found in five out of six cases. The capillaries of the necrotic areas are dilated. Fat droplets are not found here, but may be present in small numbers at the periphery of the lobules. Brownish-yellow lipoid pigments are found in the hepatic cells Some of the capillaries may contain localized accumulations of polymorphonuclears, mononuclears and plasma cells; their stellate cells become larger and detached from the walls, forming giant cells in the sinusoids and including erythrocytes and mononuclear cells in their cytoplasm. They may show vacuoles, but they do not contain fat. Sometimes their nuclei are lost. Glisson's capsule may exhibit a slight infiltration by lymphocytes, which may form nodules in association with plasma cells and mononuclears. There may be areas of necrosis here. The reticulum of the small necrotic areas is well preserved in freshly fixed material, but disappears in older tissue.

The liver of the convalescent shows wrinkling of its surface and is hard in consistence. Its section surface is congested and brownish, with distinct lobules.

Microscopically there is congestion and a variation in lobular diameters with increase in the interlobular connective tissue. Here the reticulum is increased in amount. The stellate cells are phagocytic, taking up red cells, lymphocytes and polymorphonuclears and sometimes fat droplets and coarse granular brown pigment, which gives a positive iron reaction. Yellowish-brown "wear and tear" pigment is also found to a moderate degree.

D. *Kidney:* According to Tanaka and Hayashi, there are necrotic and atrophic changes in the renal cells, especially those of the convoluted and collecting tubules. There is distension of the blood vessels and congestion of the capillaries. Tanaka found yellowish crystals of calcium oxalate and casts.

Our material, obtained from six cases during the defervescent period shows about the same changes in each case. Macroscopically the capsule is easily stripped, the stellate

veins distinct, and the parenchyma somewhat soft. The cut-surface shows a more or less swollen and cloudy cortex, congested medulla and a few hemorrhagic areas in the pelvic mucosa.

Microscopically, it is seen that the epithelium of the convoluted tubules is swollen, irregular, and granular; it may be vacuolated. Its nuclei are at times shrivelled and poorly stained, at others entirely gone. The convoluted tubules are dilated and filled, in places, with granular or homogeneous materials. Some portions of the Henle's loop and straight tubules were also filled with granular substance. Bowman's capsule is dilated and filled with fibrinous and granular material. No fatty changes are noted in the epithelium of the convoluted tubules. The medullary capillaries are dilated and, in places, contain accumulations of lymphoid and plasma cells, and lymphocytes. Some of these cells were phagocytic for red cells and lymphocytes.

Some specimens showed a perivascular plasma cell and lymphocytic infiltration.

The kidney of the convalescent showed an increased consistence, a more or less swollen and grayish cut surface, and under the microscope it was seen that some of the nuclei of the tubular epithelium were lost and the lumina of the convoluted tubules had become dilated and often filled with granular material.

E. *Heart:* Reports on the heart represent the cardiac muscle as pale gray, relaxed and cloudy. The examination of our six cases obtained during the stage of defervescence, shows that the right ventricle is slightly dilated but the size of the heart as a whole not increased. Its consistence is normal. The cardiac muscle is slightly opaque. The content of the right ventricle is a moderate amount of liquid blood.

Microscopic Examination: There is more or less brown pigment at the nuclear poles. In two cases the muscle shows segmentation and fragmentation. There is no fatty infiltration. Sometimes there is round-celled infiltration around the vessels of the myo-and endocardium, the cells being lymphocytes and plasma cells. The blood in the large veins does not coagulate, remains fluid and is dark red.

F. *Lungs:* Macroscopic Examination: The lungs are congested and there are often hemorrhagic areas on the pleura. Sero-sanguinous fluid may be squeezed out of the organ on pressure. In one case out of six there was a pneumonic area in the right upper lobe, but no signs of pneumonia were found in the other cases. In one there is superficial hemorrhage. The bronchial mucosa in the majority of cases is congested and shows catarrhal inflammation.

Microscopic Examination: The pulmonary vessels are congested and the alveoli in some places filled with granular serum. In some areas there are enough cells in this to constitute an early pneumonic exudate, of the lobular type. In the case with a pneumonic area, the alveoli are filled with fibrin and mononuclear cells. Punctate hemorrhages were microscopically demonstrable in half of the cases. The bronchial mucosa is covered with mucus and cellular elements and debris. There is an increase in lymphocytes, and plasma cells are noted.

G. *Digestive System:* The gastric mucosa of two out of the six cases examined is grayish-white, shows an increased mucus secretion, and small punctate or linear hemorrhages. Histologically, there is a round cell infiltration of the mucosa, with increased numbers of plasma cells. The submucous blood vessels are somewhat dilated.

The intestinal mucosa, macroscopically, shows occasional patches of hemorrhage, otherwise it is unchanged. The intestinal lymph follicles are not hypertrophied. Histologically, there is an increase in the number of plasma cells in the mucosa, but this is only occasionally seen in the case of the agminated nodules. There is congestion of the vessels of the submucosa and some of the capillaries contain lymphocytes, plasma cells and large vesicular cells, some of which are phagocytosing lymphocytes and red cells. The plasma and vesicular cells are also seen among the connective tissue fibers of the tract, the vesicular cells showing phagocytic proclivities in this situation also.

H. *Suprarenal Glands:* The cortical fat is decreased and only little remains in the zonae fasciculata and reticularis. There is isolated necrosis of the cortical cells. Some of the

material shows areas of round cell infiltration of both cortex and medulla, with plasma cells among the invading cells.

I. *Nervous System:* No change is noted in the cortex or white matter of the cerebrum or cerebellum; there is an accumulation of mononuclear round cells in the posterior hypophyseal lobe, a part of them being plasma cells.

J. *Summary:* A review of the changes found in the various viscera shows that there is no specific visceral change in tsutsugamushi disease. As a rule there are the general lesions of septicemia and one notes a resemblance of the pathological picture with that of typhoid. Macroscopically the spleen and liver are swollen, the parenchymatous organs show cloudy swelling. Microscopically we find focal necroses, round-cell infiltrations and increase in the reticulo-endothelial elements. The latter appear as vesicular cells with marked phagocytic propensities and are especially numerous in the spleen, bone marrow and liver, sometimes being found in the vessels of other organs. The round cell infiltration is sometimes nodular and it is possible to demonstrate transformations from lymphocytes to plasma cells. These are increased more or less in every organ. Cellular necrosis is observed in the parenchyma of various organs, and may go on to form miliary necroses which are most often seen in the spleen, liver, and bone marrow. Dissociation of the parenchyma of the liver is also typical. Congestion of the viscera is universally noted and hemorrhages are occasionally seen; they may be found most commonly as microscopic areas of bleeding in the lung. No marked changes are found in the pancreas, thyroid and reproductive glands.

8. Pathological Changes in the Fetus.

The lesions noted in three fetuses examined, are milder in degree, as compared with the foregoing cases. There is, however, an increase of vesicular and plasma cells in the spleen, lymphnodes and bone marrow. The vesicular cells are phagocytic here, as in the adults. There is no marked necrosis.

The hepatic cells of the third case contain a dark-brown iron-containing pigment. There are small foci of necrosis,

with local accumulations of leukocytes and the sinusoids are congested. Some of the epithelial cells of the convoluted renal tubules show loss of nuclei and vacuolization. The capsular space in the glomeruli is filled with an homogeneous material. The epithelium shows no fatty infiltration. The other organs are not changed.

Blood obtained from the umbilical vessels was injected into animals, but we were unable to demonstrate the presence of tsutsugamushi disease virus. The spleen of the first case was emulsified and likewise injected into animals about two or three hours after the patient died, but the result was quite negative. The third fetus was suspected of having congenital syphilis, but the lesions found at necropsy, when compared with those in the adults, left no doubt as to its death having been due to tsutsugamushi disease. Thus this series of fetal cases indicates that the virus of the disease is not transmitted to the fetus through the placenta, but the toxin may be absorbed from the maternal blood-stream. There is no question as to the specificity of the changes found in the fetus.

CHAPTER III.

EXPERIMENTAL TSUTSUGAMUSHI DISEASE IN ANIMALS.

It is very important to secure the most susceptible experimental animals when studying the etiology of any febrile malady of unknown origin and we have used a large variety in studying tsutsugamushi disease. We injected the blood of patients, or of monkeys infected with this virus, into all sorts of animals and followed their subsequent behavior, taking their daily temperature in most instances and examining their blood, the appearance of leukopenia being particularly noted. Their clinical symptoms were also carefully observed, whether they were typical of the disease or not. Besides this, necropsies were performed in the case of the smaller animals, in various stages of the experiment. Thus far we have used anthropoid apes, Japanese and various South Sea Island monkeys, rabbits, guinea pigs, white rats and field voles, mice, dogs, cats, goats, calves, horses, pigs, chickens, geese, pigeons, and marsh-birds.

Of all these animals only the orang-utan and the various monkeys, developed fever and altered blood-pictures that indicated that they had contracted the disease. Among the other animals, the guinea pig, field vole, white rat, and occasionally the rabbit all harbored the virus in their system without showing any symptoms of the disease; but if their emulsified spleen, or their blood were injected into other, more susceptible animals, these would develop tsutsugamushi disease. The other animals enumerated above showed no reaction whatever.

1. Orang-utan.

Kitashima and Miyajima (81 - 84) used one orang-utan in their studies and reported that it developed typical fever following the injection of blood from a patient with tsutsugamushi disease, but they failed to find any eruption and did not report any blood examination. We used two orang-

utans *(Pithecus satyrus)* in 1919, which were born in Sumatra and, according to the dealer, were four years old. Before experimenting upon them we took their temperature, examined their stools for parasites, measured their hemoglobin, and made differential and total counts of their blood cells. Unfortunately orang-utan No. 2. died at this point in the experiment from acute catarrhal enteritis.

We then took 4 c. c. of one-fifth citrated blood from our monkey No. 157, which had developed tsutsugamushi disease eight days after being injected with blood from a human case, and we injected it into the surviving orang-utan. Fever came on eleven days after the injection. At first intermittent, it reached a maximum of 40.2° C. on the thirteenth day when the ape died. After the onset of fever it had lost its appetite, and its weight had fallen from 4,350 gm. to 3,330 gm. a loss of 1,020 gm. Seven days after the onset there appeared two pale red papules upon the upper lip which was somewhat pale. The margins of these papules were not sharp and the eruption disappeared three days later. No other area of exanthem could be found after a careful examination.

During the course of the disease the changes in the blood picture were carefully noted. The stools of both apes contained no parasites. At first the physiological standards of blood count, differential count, and hemoglobin were determined, the examinations being repeated at intervals in the first animal during the course of its illness. The results of the examinations of the blood of orang-utan No. 2 were charted before it died of enteritis. The normal blood picture of the orang-utans is as follows:

Erythrocytes per c. mm. (avg. of nine counts): 4,420,000. Extremes: 4,390,000 and 4,480,000.

Hemoglobin (avg. of ten determinations) 58%. Extr. 53% - 61%.

Leukocytes per c. mm. (avg. of twelve counts): 9,400. Extremes: 8,000 - 12,000.

Differential counts of the white cells in percentages are as follows: (Avg. of five counts) Neutrophils, 48.5% (33% - 59.4%) Lymphocytes, 43.0% (29.2% - 60.4%), Eosinophils, 5.1% (1.5% - 10.5%) Transitionals and large mononuclear cells, 3.6% (0.6% - 7.8%).

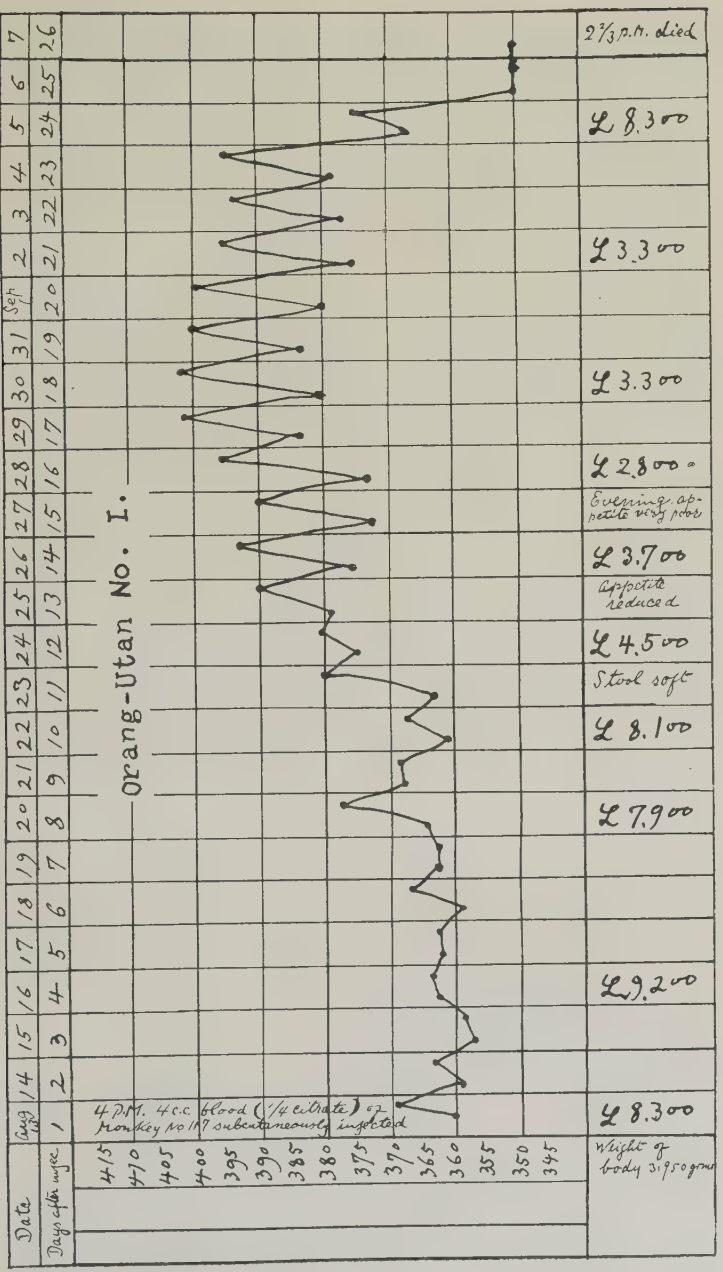

Orang-Utan No. I.
Date
Days after injec.
Aug
Sept
4 P.M. 4 c.c. blood (1/4 citrate) of monkey No 147 subcutaneously injected
£8.300
£3.300
£3.300
£2.800
Evening appetite very poor
£3.700
Appetite reduced
£4.500
Stool soft
£8.100
£7.900
£9.200
£8.300
2 1/3 p.m. died
Weight of body 3.950 grms

Figure 14

A comparison of these figures with those of orang-utan No. 1, after injection of the virus, shows that there is a slight decrease in the number of erythrocytes during the first period of the disease. On the fourth day they decreased to 3,940,000, the leukocytes also showing a slight decrease. On the eighth day the hemoglobin was 50% (Sahli). The most pronounced changes were seen in the case of the leukocytes, not only the total number, but the differential percentage being altered. (Fig. 14).

There is a marked decrease in the number of leukocytes after the onset of the disease. On the sixth day there were 2,800, about one-third the normal count of 8,200, and from then on the count gradually increased until, at the time of death (the thirteenth day), it had reached the original figure.

There is some diversity in the normal differential count of the two apes, but there appears to be an absolute, as well as a relative decrease in the number of lymphocytes during the fastigium. On the other hand, there is a decrease in the absolute, and an increase in the relative number of the neutrophils. Later, however, there is lymphocytosis and a neutrophil leukopenia. The eosinophils gradually decrease and entirely disappear by the tenth day of the illness. They did not reappear by the thirteenth day and the death of the animal rendered further examination impossible. There was no marked change in the number of transitionals and large mononuclears.

The foregoing blood findings are entirely in accord with those observed in the Japanese monkey, so that we may safely conclude that the orang-utan inoculated with the virus obtained from patients and animals contracted tsutsugamushi disease. This assumption is strengthened by the typical temperature curve of the disease.

This orang-utan died thirteen days after the onset of the disease and a necropsy was immediately performed.

Necropsy on Orang-utan No. 1.

Body: Is that of a greatly emaciated orang-utan, its subcutaneous fat decreased in amount and yellowish. The skeletal muscle is light red and dry. There is no abnormal fluid in the abdominal cavity. The omental fat and that of the visceral capsules is well preserved and yellow. The stomach is distended, but the intestines not particularly so.

Organs: Heart: Flaccid and contains fluid blood. The myocardium is slightly clouded, the endocardium and valves are normal. One *Filaria immitis* is found in the right ventricle. The aorta is normal.

Lungs: Both organs have two lobes and are pale gray. There is nothing abnormal about the lungs, pleural cavities, or pleura. The mucosa of the bronchi and trachea is pale.

Spleen: 21 gms. The capsule is tense and indurated, the cut surface dark red, the splenic pulp is difficult to scrape away, the follicles are distinct. (The spleen of the other orang-utan weighed 11 gms.)

Liver: 110 gms. The capsule is smooth, the consistence of the organ is normal, its color slightly yellowish. The cut surface appears clouded and the lobules are indistinct. The bile ducts and portal vein are normal. The gall bladder is filled with thick, dark brown bile and its mucosa is normal.

Gastroenteric Tract: The stomach contains some coagulated milk and its lining is coated with thin mucus. The intestine contains whitish feces, less abundant in the ileum. The upper portions show an increase in mucous secretion. The colon contains soft, whitish feces, its mucosa is pale and free from mucus secretion. The mesenteric fat is slightly increased in amount.

Pancreas: This is normal in appearance.

Suprarenal Glands: There is a small amount of medulla and the cortical fat is indistinct.

Kidneys: These are not grossly remarkable.

Lymphnodes: The mesenteric nodes are slightly swollen, but soft; the axillary nodes are also slightly swollen, flat and soft; the cervical nodes are only slightly enlarged in their lower groups, in the supraclavicular fossa; the nodes about the pancreas are also slightly swollen, but soft; the femoral nodes are small.

Histological Examination: This shows marked changes in the blood-forming organs: the spleen, lymphnodes, and bone marrow. The spleen is congested, its pulp infiltrated with erythrocytes and its follicles somewhat encroached upon. Splenic cells are more or less increased in number, plasma cells are occasionally found, and a few giant cells, containing erythrocytes and other cells, are seen. There are no necrotic areas and no fat.

Several lymphnodes were taken from various regions and examined microscopically. The reticuloendothelial cells are more or less numerous, plasma cell production is distinctly demonstrable, and there are no areas of necrosis.

The cellular elements of the bone marrow are more or less increased in amount; there is an increase in lymphocytes and myelocytes, a few giant cells containing erythrocytes and other cells are found; there is a production of plasma cells, but no necrosis is noted.

There are no changes in the parenchymatous organs; liver, kidneys, or pancreas. The lungs are normal except for slight congestion. The myocardium is normal. There are a few phagocytic cells in the supporting tissues. Fatty infiltration is not noted in any of the organs. There is some decrease of cortical fat in the suprarenal glands. The cerebrum is normal. No histological changes are found in the stomach or intestines.

The marked gross and microscopic changes in orang-utan No. 1 are the following. Macroscopically there is swelling of the spleen and of the systemic lymphnodes; microscopically one sees cellular increase, especially of plasma cells and the appearance of phagocytes containing erythrocytes and other cells. These changes correspond with those found in tsutsugamushi disease, the only difference being the lack of definite degeneration and necrosis.

As to what killed the animal; no definite cause of death was found, but clinically there was high temperature and distinct leukopenia. These findings lead us to suppose that tsutsugamushi disease killed the ape, but the pathological lesions were very mild. On the other hand, there are no clinical or pathological changes upon which to base any other definite diagnosis. It is very probable that there was a general intoxication, in this case, that acted chiefly upon the vasomotor apparatus and interfered strongly with the function of the heart.

No definite statement can be based upon these results, as we experimented with only one orang-utan, but if we consider the symptoms that follow the injection of tsutsugamushi disease virus into this animal, we may say that it is probably more susceptible to tsutsugamushi disease than are the lower monkeys. Although orang-utans are very high priced and one can not obtain them in large numbers at one time, the experimental results obtained are so much more accurate than they are in the case of other monkeys that the use of such expensive animals is justified in the study of the etiological factor of this disease. Even so, we did not find any typical rash anywhere on the animal's body, excepting the two doubtful papules on the lip.

2. Japanese and other Macaques. (Figures 15, 16, 17, 18).

Various species of monkey were used, chiefly several hundred Japanese monkeys *(Macacus fuscatus, Blyth)*, twenty to thirty Java monkeys *(M. cynomolgus, M. aureus)*, several *M. cyclopis*, and one *M. remetrinus*. Of all these, those that had symptoms distinct enough to enable us to make a diagnosis of tsutsugamushi disease, were *M. fuscatus* and *M. cynomolgus*. Since the *M. fuscatus* is more readily procurable and indigenous in Japan, we chiefly used that species. In order to infect monkeys artificially with the disease, 3 to 4 c. c. of patients' blood containing one-fourth of 2% sodium citrate solution should be injected; to infect them naturally they are brought into the endemic region and exposed to biting akamushi.

When a monkey is bitten by a virus-bearing akamushi a bite-lesion, similar to the primary lesion in the human, is developed; and, as noted in the human, the disease that follows has a definite incubation period and a fever with leukopenia, but no eruption. Monkeys rarely succumb to the disease and after the fever has subsided, they are found to have developed

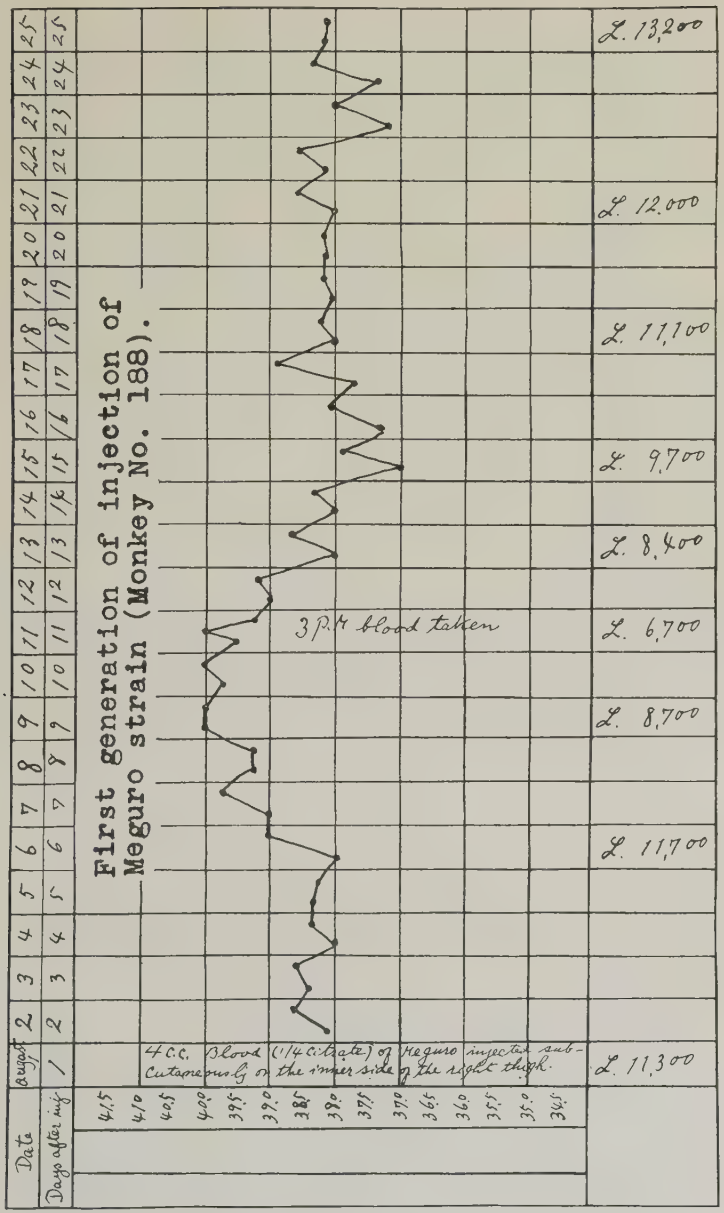

First generation of injection of Meguro strain (Monkey No. 188).
Date
Days after inj.
4 c.c. Blood (1/4 c.c. late) of Meguro injected sub-Cutaneously on the inner side of the right thigh.
3 P.M. blood taken
L. 11,300
L. 11,700
L. 8,700
L. 6,700
L. 8,400
L. 9,700
L. 11,100
L. 12,000
L. 13,200

Figure 15

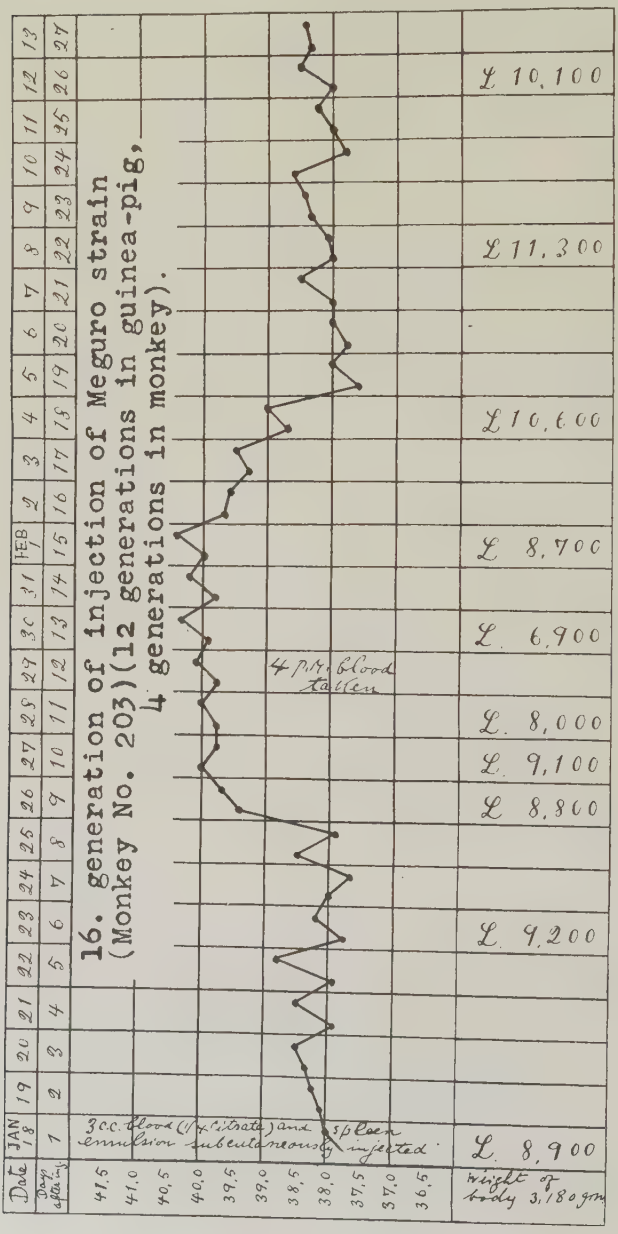

16. generation of injection of Meguro strain
(Monkey No. 203)(12 generations in guinea-pig,
4 generations in monkey).
3cc. blood (Peritrate) and emulsion subcutaneously
spleen injected.
4 p.m. blood taken
Date
Days after inj.
JAN 18 19 20 21 22 23 24 25 26 27 28 31 FEB 2 3 4 5 6 7 8 9 10 11 12 13
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
41.5 41.0 40.5 40.0 39.5 39.0 38.5 38.0 37.5 37.0 36.5
£ 8,900
£ 9,200
£ 8,800
£ 9,100
£ 8,000
£ 6,900
£ 8,700
£ 10,600
£ 11,300
£ 10,100
Weight of body 3,180 gm.

Figure 16

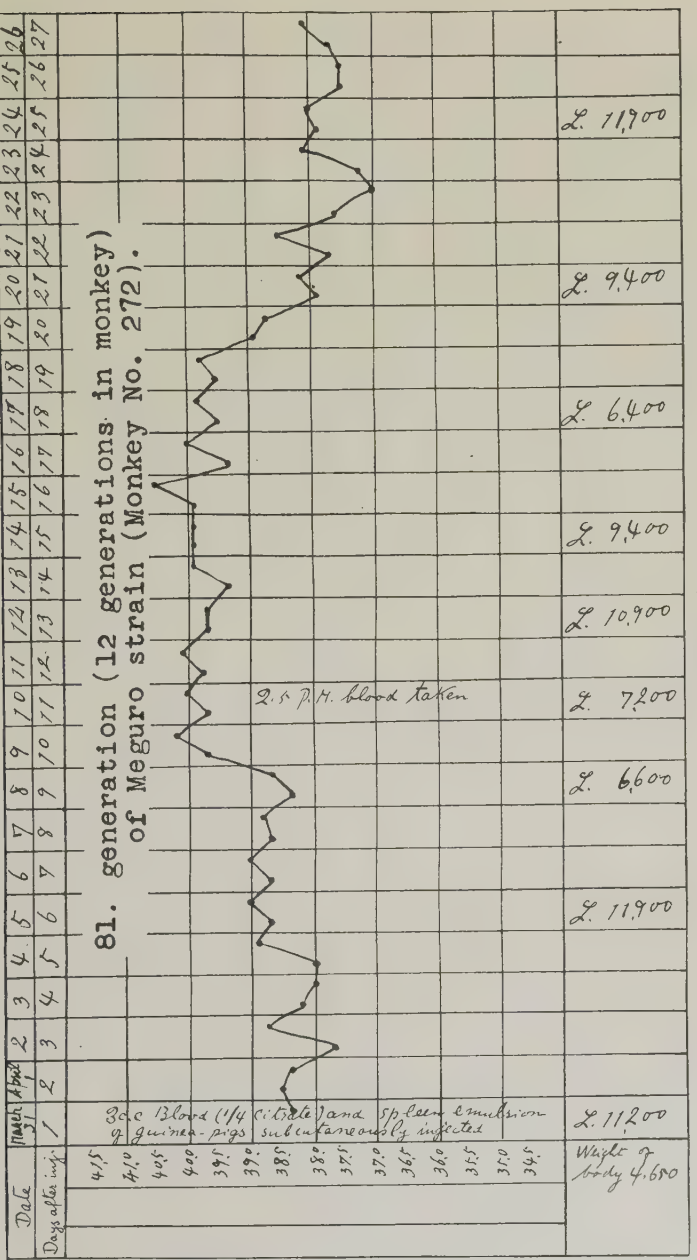

81. generation (12 generations in monkey) of Meguro strain (Monkey No. 272).
Date
March 31
April 1
Days after inj.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
4.5°
4.0°
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
2.5 c.c. blood (1/4 citrate) and spleen emulsion of guinea-pigs subcutaneously injected
2.5 P.M. blood taken
L. 11,200
L. 11,900
L. 9,400
L. 6,400
L. 9,400
L. 10,900
L. 7,200
L. 6,600
L. 11,900
Weight of body 4,680

Figure 17

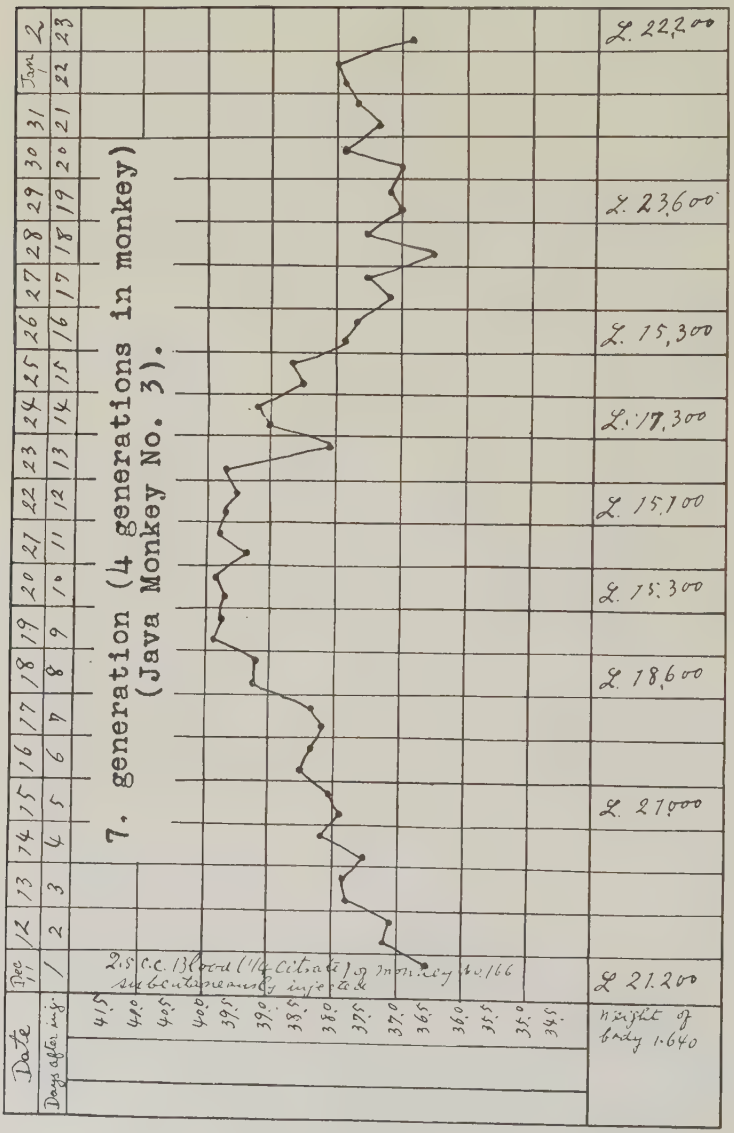

7. generation (4 generations in monkey)
(Java Monkey No. 3).
Date
Dec. 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 Jan. 2
Days after inj. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
2.5 c.c. Blood (1/4 Citrate) of Monkey No. 166 subcutaneously injected
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
L. 21,200
L. 22,200
L. 23,600
L. 15,300
L. 17,300
L. 15,100
L. 15,300
L. 18,600
L. 21,000
Weight of body 1.640

Figure 18

a definite immunity. Whether or not a monkey had contracted the disease was determined by the character of the fever curve, the blood picture, and immunity tests.

Susceptibility: We used several hundred monkeys for our experiments with tsutsugamushi disease and eight strains of virus; five human strains, one of which was Formosan; one field vole strain; and two from akamushi. Out of fifty-one monkeys, which had never been treated in any way before, only three were insusceptible and these three were subjects of experimentation with the Formosan strain. All the monkeys inoculated with the local strains were susceptible. We may, therefore, conclude that the Japanese monkey, *M. fuscatus,* is suitable for the purpose of studying tsutsugamushi disease experimentally.

Symptoms: All the monkeys contracting the disease showed the typical fever curve. The incubation period of thirty-two monkeys inoculated with the local strains was between three and thirteen days, the usual extremes being eight to nine days, the average 8.6 days. One case, inoculated with the akamushi strain, had an incubation period of nineteen days, which is exceptional.

The duration of the fever was five to fifteen days, the usual extremes being seven to twelve, the average 9.7 days. The normal temperature for these monkeys is 38.0° - 38.5° C. In two or three days the fever reached its high level, being maintained at 39.5° - 40.0° for from five to eight days, after which it gradually fell as in the human cases. Its course was shorter in the monkeys. With the Formosan strain, sixteen monkeys showed incubation periods of from nine to thirteen days (in one case only six), the average being 10.3 days. (Figs. 19 and 20). The duration of their fever was between five and thirteen days, the usual extremes eight to ten days, the average ten. The course of this fever was, however, different from that caused by the local strains: it had two fastigial periods, the first reached in one or two days with a temperature of 39.0° or 39.5°, lasting four or five days; and the second following this with a fever plateau of 40.0°, which was maintained for two or three days, after which the temperature gradually subsided. During the period of fever the animal was more or less quiet and its appetite diminished. As a rule there was no diarrhea, although

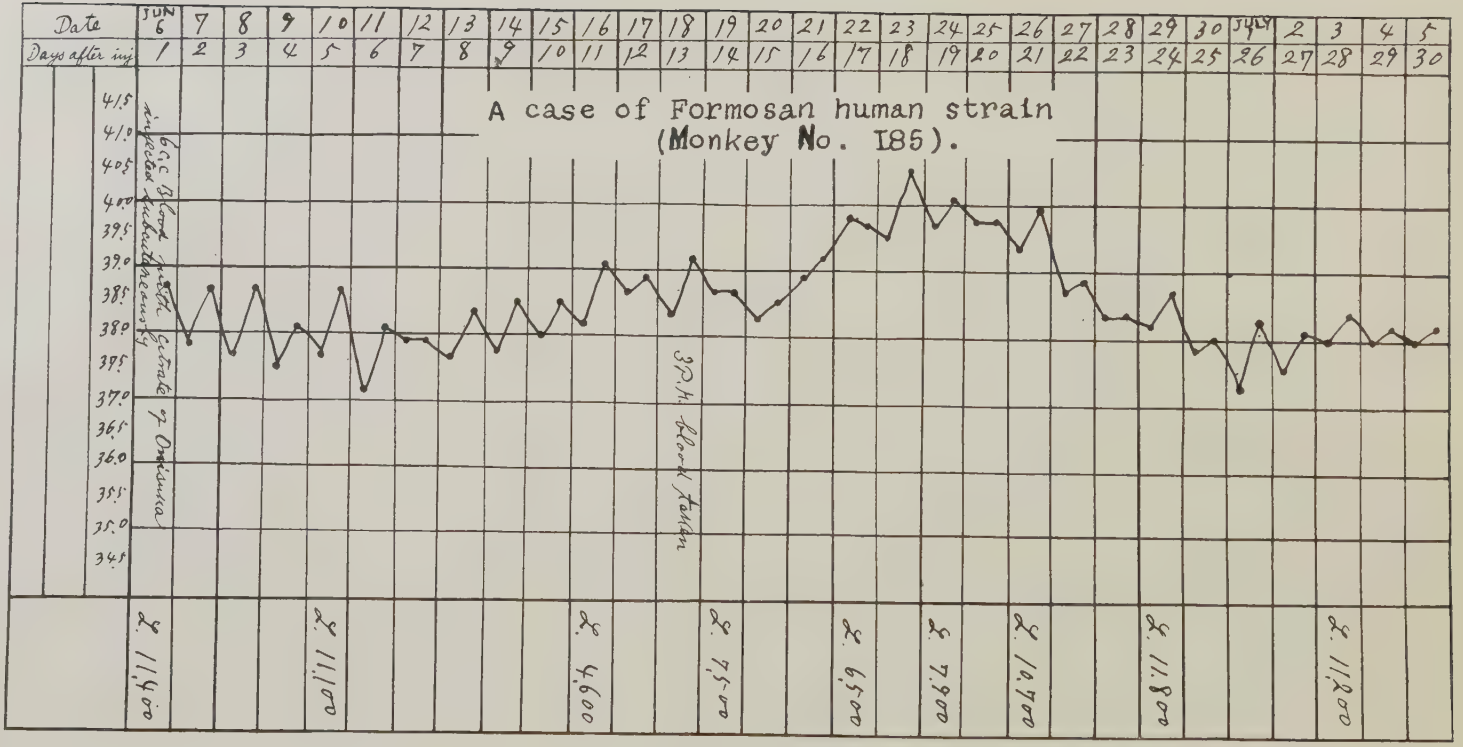

A case of Formosan human strain
(Monkey No. I85).
Date
Days after inj
JUNE 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 JULY 2 3 4 5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
3 C.C. Blood injected subcutaneously
1/2 Oxalate
37 C.C. Blood taken
♂ 11,400
♂ 11,100
♂ 4,600
♂ 4,500
♂ 6,500
♂ 7,900
♂ 10,400
♂ 11,800
♂ 11,200

Figure 19

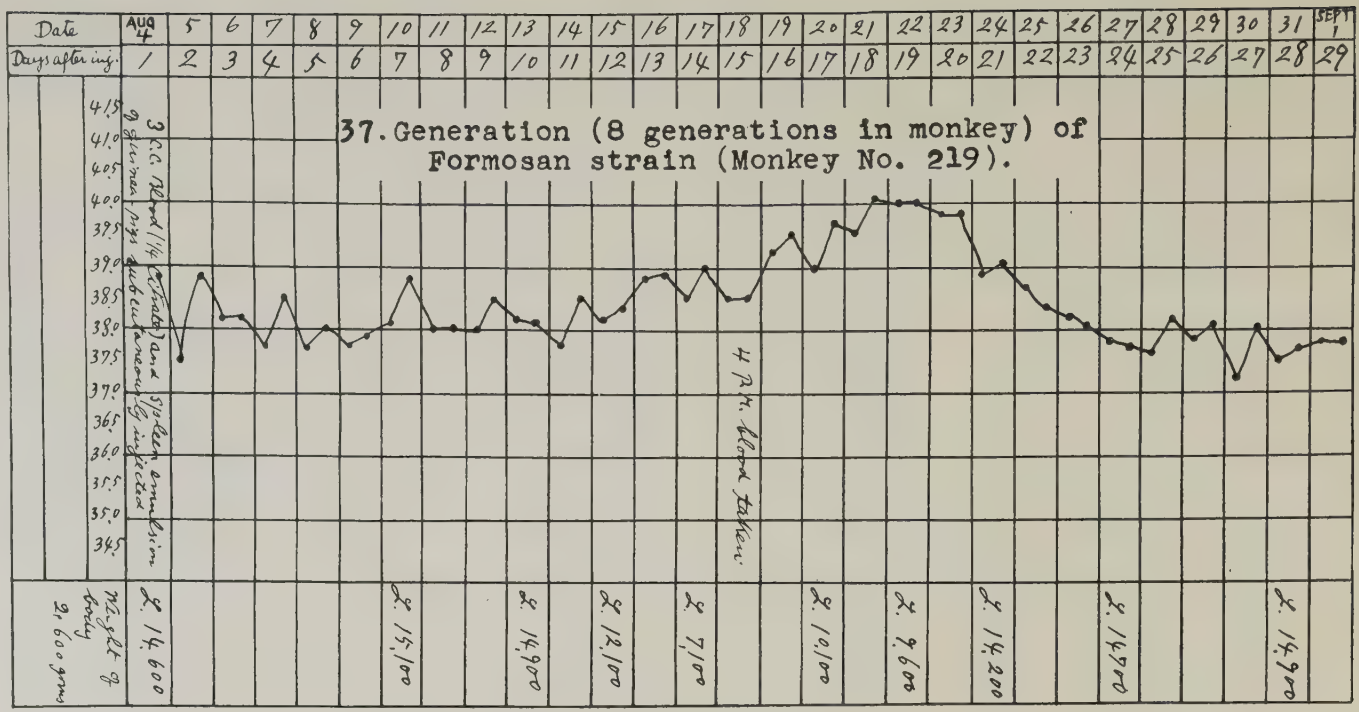

37. Generation (8 generations in monkey) of Formosan strain (Monkey No. 219).
Date
Days after inj.
AUG 4
SEPT 1
3 C.C. blood (1/4 citrated) and spleen emulsion
2 animals dying with acute anemia after injection
4 P.M. blood taken.
Weight & body
2,600 grms.
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5

Figure 20

with certain strains there was slight diarrhea before, or during the febrile period, lasting two or three days. The body-weight was, as a rule, decreased; in the fastigium from 120 to 500 gms. or an average of 170 gms. were lost.

Immunity: A monkey that has once contracted the disease acquires a definite immunity which, according to our experience lasts from two to three years.

Blood Pictures: The blood pictures of monkeys with this disease are very typical and afford a basis for diagnosis, but before describing them in detail, we will review that of the normal monkey.

A. Blood Picture in the Normal Monkey.

There were no accounts of normal blood-picture of the Japanese monkey until we reported it in 1914, since then two other investigators have done so.

B. Changes in the Blood Picture of Japanese Monkeys with Tsutsugamushi Disease.

We have made several reports since 1914 on the changes in the blood picture of monkeys resulting from the injection of blood from tsutsugamushi disease patients, or that of monkeys infected with the disease. The changes noted in the case of the leukocytes is the most noteworthy.

a. Leukocytes: Out of forty-eight monkeys, never before treated in any way, five had a leukocytosis before the onset of fever (an increase of 600 - 5,300), while twenty-four showed a leukopenia (a decrease of 800 - 6,600). No changes were noted in the remaining nineteen. After the onset of fever, however, all the monkeys had a leukopenia which was most intense in the middle of the fastigium, when the count fell from four-fifths to one-third of normal. The reduction in the majority of cases was from two-thirds to one-half normal. The leukocytes gradually increase in number from this time on and in twenty-three monkeys this increase reached a maximum in one to four days before the disappearance of the fever, while in the other twenty-five this occurred in one to eight days. In twenty-seven monkeys the leukocyte-count exceeded that made at the time of inoculation and thus they had a post-infectious leukocytosis. In all cases there was a typical leukopenia during the fastigium.

Polymorphonuclear neutrophils: In typical cases the decrease in the number of these parallels that of the total leukocyte count. Their relative percentage is increased from the outset and before the leukopenia reaches its lowest figure the percentage may be as high as 51 -- 60% At the height of the leukopenia the percentage begins to decrease, falling nearly to normal and going below normal by the end of the third stage of the disease. From then on the number of polymorphonuclears gradually increases and during convalescence it becomes nearly normal. The absolute number of polymorphonuclears shows a marked decrease during the fastigium, but this decrease reaches its maximum more slowly than does the relative percentage. During defervescence it increases and reaches normal during convalescence.

Lymphocytes: The relative number of lymphocytes already shows a decrease before the onset of fever and, coincident with this, it falls still lower, the percentage reaching 22% - 42%. When the leukopenia is most marked the number of lymphocytes begins to increase and in the third period of the disease, when the polymorphonuclears are at their minimum, the percentage of lymphocytes is highest, producing a relative lymphocytosis. Some cases showed as high as 59%. After reaching a maximum the number gradually falls to normal. The absolute lymphocyte count shows a decrease from the beginning and, shortly before the maximum leukopenia is seen, it reaches its maximum. It then gradually increases to become an absolute lymphocytosis, finally falling to normal.

Immature Leukocytes: During the febrile period metamyelocytes and myelocytes appear in the blood, the latter being most numerous during the fastigium.

Other Leukocytes: Eosinophils are decreased during the period of fever, particularly the fastigium, but they do not disappear entirely as in the case of man. Their number is gradually increased, but there are no instances of eosinophilia. There are no marked changes in the basophils, nor in the case of the transitional and large mononuclear leukocytes. In some cases Arneth's stimulative types may appear during the fever.

b. Erythrocytes: Polychromatophilia, and an increase in the basophilic granulation of these cells may often be seen during the fastigium and in the third period, but since these phenomena are also found in the blood of normal monkeys their appearance in that of inoculated animals may not be considered as abnormal. Normoblasts also may be seen occasionally during the febrile period, but there are no other morphological changes in the red cells. Their number is decreased more or less during the fastigium. The hemoglobin shows no change during the entire course of the disease, except that during the leukopenic phase it may be slightly decreased.

Similar blood pictures are noted in *M. cynomolgus* (Java monkeys). In all cases the change in the blood picture is most distinct in the case of the white cells; there is marked leukopenia in the fastigium and it is due to the decrease in the relative and absolute number of lymphocytes. The absolute and relative number of polymorphonuclears show some increase. Post-infectious leukocytosis is noted in about one-half the cases, but in this period there is an absolute and relative lymphocytosis. The curve of the lymphocytes crosses that of the polymorphonuclears. The acidophils may be decreased, but do not disappear entirely. There is no post-infectious eosinophilic leukopenia.

Kodama's results, obtained from the examination of the blood of tsutsugamushi monkeys, agree with ours on the whole.

A comparison of the foregoing data with those from human cases shows an entire accord. The leuko-or lymphopenia is always found during the fastigium in this disease and it is the most important symptom thereof. The above blood pictures, together with the fever-curve, determine whether or not a monkey has contracted the disease.

C. Pathological Findings in the Japanese Monkey, (Macacus fuscatus).

This monkey is the only suitable animal for the study of the etiology of tsutsugamushi disease. The fever curve and blood-findings, especially the leukopenia, and the immunity test are all determinative points which show whether or not

the animal has contracted the disease. The symptoms are far milder in monkeys than in man. So long as the etiology of tsutsugamushi disease has not been determined, it is absolutely necessary that it should be studied from all angles; therefore pathological examination of the infected monkeys, for comparison with that of human beings, and the establishment of a sequence of definite pathological changes, are necessary for the determination of the nature of the disease.

Kitashima and Miyajima, and Hayashi, have reported the results of the examination of bite-lesions and lymphnodes in the Japanese macaque, infected either by the natural bite of akamushi or artificially, by the injection of blood from patients. I also compared the changes noted in monkeys with those in the human at the fourth meeting of the Japanese Pathological Society in 1913. The following description includes additional facts that were subsequently established. We had eight monkeys infected by the subcutaneous injection of blood from patients or from monkeys. These animals were killed at various stages in the disease. Besides these eight, a ninth monkey died of the disease and was necropsied. Of the eight monkeys, two were killed three days, one four days, and two twenty-one days after the onset, one fourteen days after inoculation, and one two days along in the convalescent period. The ninth monkey died of the disease on the sixteenth day after the onset of symptoms. Besides these nine cases, there were six in which the macaques died of severe diarrhea between the fifth and sixteenth days of convalescence.

Description of Gross Lesions: The spleen is swollen in all cases and weighs between seven and sixteen grams, its normal weight being 3 - 7 gm. The capsule is tense, the cut-surface dark-red and the pulp soft enough to be scraped off; the follicles may be recognized, but they are somewhat soft. The lymphnodes, both regional and systemic, are swollen, their cut surface shows medullary congestion. There are no macroscopic necrotic foci. As for changes in the other organs there is nothing of note save cloudy swelling of the parenchymatous viscera.

Description of Histological Lesions: The follicles of the spleen are distinctly compressed, only some of them showing "germinal centers" and a few contain deposits of hyalin material. The sinuses are distended and the pulp filled with blood. The cells of the reticuloendothelium are somewhat increased in number, contain vesicular nuclei and show mitotic activity. A variable number of large cells with inclusion of erythrocytes, lymphocytes and plasma cells, or nuclear fagments, is found

in every case. In a few instances brown pigment is present. The plasma cells are much increased in numbers and in the neighborhood of the follicles there is ample evidence of their transformation from lymphocytes. As to retrograde processes; there are no necrotic foci, but cellular destruction and nuclear degeneration are everywhere present.

The lymphnode sinuses are dilated and filled with large cells, rich in cytoplasm and containing lymphocytes and plasma cells. These large cells often contain basophilic granules, or become vacuolated and phagocytic. In the follicles and follicular cords the lymphocytes are decreased, while the plasma cells and reticulum cells are increased in number. Some of the latter exhibit phagocytic activity. The transformation of lymphocytes into plasma cells is, here too, everywhere present. In some specimens there is karyorrhexis.

The bone marrow is congested; myelocytes are more or less increased, lymphocytes are few, but plasma cells are present in abundance, occupying the perivascular spaces. Only a few large phagocytes are observed, taking up erythrocytes, plasma cells and lymphocytes. Cellular degeneration and karyolysis are seen here and there.

The liver shows a few minute foci of necrosis, there is destruction of the hepatic epithelium and disruption of the trabecular arrangement of its cells often occurs. This is particularly marked around the central veins. Glisson's capsule is often infiltrated with round cells, mostly of the plasma cell type. The Kupffer cells are not visibly altered. The degree of fatty infiltration of the hepatic epithelium is not uniform in all instances.

In the kidney the epithelium of the convoluted tubules shows local karyolysis or karyorrhexis; there is no fatty infiltration. A distinct perivascular round cell infiltration is seen in the intertubular spaces of both cortex and medulla, plasma cells being a prominent constitutent of the exudate.

The myocardium shows no fatty infiltration, nor any marked change. In a few cases there is slight cellular infiltration between the muscle fibers, which is shared in by plasma cells.

As to changes in the other organs. There is more or less increase in the number of round cells in the peribronchiolar tissue of the lungs and plasma cells are found among them. In a few cases there is an increase of round cells in the meninges. The fatal case showed petechial hemorrhages in this situation. Karyolysis may be seen in the cells of the cerebral cortex. The stomach, in the large majority of cases, shows no particular change, but in one there was an increase in the number of plasma cells in its mucosa. The intestinal mucosa shows a similar increase in plasma cells, but they are not noticeable in the intestinal follicles. The secretion of mucus is more or less increased. There is a cellular increase in the tonsils, which show an increase in subepithelial plasma cells in some instances. The thyroids, salivary glands, pancreas, gonads, uterus and bladder present no particular lesions.

Summary of the Pathological Findings:

A brief summary of the foregoing findings follows. The most noteworthy progressive change is the increase in the number of cells, which is most marked in the hematopoietic system, but may also be seen in the parenchymatous organs.

Thus we note changes in the liver and kidney and, very occasionally, in the heart and the digestive tract. As in human cases, the cellular increase chiefly affects the macrolymphocytes and plasma cells and some of the former show phagocytic activity. Of the retrograde changes, necrosis is very slight when compared with that in human cases, but very small foci may occur in the liver, and sometimes in the lymphnodes. Cellular necrosis is, however, common. In the parenchymatous organs, especially the liver, there is irregularity of the arragement of the parenchyma. The changes found in the Japanese monkey coincide histologically, as well as macroscopically, with those found in human cases, the only difference being the comparative mildness of the lesions in the monkey.

A few words should be said concerning the changes noted in the tissue at the site of inoculation. The induration caused by the injection of the virus into the subcutaneous tissues subsides in two or three days, and there are no further local consequences. Nagayo has reported, however, that if the injection be made intradermally a papule forms, changing into a vesicle that closely resembles the akamushi bite lesion. I confirmed the experiment by injecting 0.001 to 0.002 c. c. of virulent blood into the corium of three monkeys. Two of these showed red papules at the injection site, one of which later became pustular. A crust formed over the lesion and it was finally absorbed. The lesion was removed and when examined histologically a cellular exudate similar to that in human cases, was found, the center was necrotic and there was marked nuclear destruction. There was a large number of large mononuclear cells and only a few polymorphonuclears. The granular layer of the epidermis was thinned-out and there was parakeratosis.

Thus, when tsutsugamushi disease virus was injected intradermally, certain typical changes were produced, just as though the akamushi had bitten the skin. Therefore the lesion produced at the site of the bite of an akamushi bears no relation to the sucking tube of the insect, but is caused by the virus of the disease that is mingled in the secretion of the mite.

3. Pathology of the Disease in Other Laboratory Animals.

When a guinea pig is injected subcutaneously with 1 c. c. of one-quarter citrated blood from a tsutsugamushi disease patient, or from an infected monkey, there is no rise in temperature, as a rule, nor leukopenia. The Formosa strain, however, sometimes produces fever. There is also no loss of weight and no animals die as a result of the injection. As to pathological lesions in the guinea pig: fifteen days after the injection, or thereabout, there is merely slight swelling and occasional darkening in the color of the spleen. There is no swelling of lymphnodes nor any histological changes; but if the animal be killed thirteen to eighteen days after the injection (the optimum time being thirteen or fourteen days) and 1 c. c. of its heart's blood, mixed with an emulsion of its spleen, be injected into another guinea pig, and if this process be repeated from five to seven times and the mixture of blood and spleen from the last animal be then injected into a Japanese monkey, this animal will develop a typical attack of the disease. When, however, more than ten transfers have been made in guinea pigs, no infection will result in the monkey.

I have preserved a certain local strain of tsutsugamushi disease virus throughout eighty generations in guinea pigs and fifteen in monkeys, in this manner; the Formosan strain was passed through eighty-eight generations in guinea pigs and eighteen in monkeys. After all these transfers I found that the virulence of the strain had not been altered. The weight of the guinea pigs employed was from 300 to 500 gms. It will be seen that guinea pigs have a natural immunity to tsutsugamushi virus, but that they will harbor it in their systems. Thus we may use the cheaper guinea pig for keeping the tsutsugamushi virus alive and available, instead of wasting expensive monkeys for that purpose.

Field voles may also be used for this purpose, just as guinea pigs, but they show no clinical or pathological signs whereby one may judge whether or not they have contracted its disease. A strain of tsutsugamushi disease was repeatedly transferred in white rats and, after the thirteenth subinocula-

tion, the mixed heart's-blood and spleen of the last rat was inoculated into a Japanese monkey, which developed a typical attack of the disease. With rats, however, infection with the virus is not as certain as with guinea pigs and the presence or absence of splenic enlargement, in these animals, means nothing, as they may have enlarged spleens from other causes. Rabbits may occasionally successfully harbor the virus, but they have no symptoms of the disease by which one may be guided in their use.

CHAPTER IV.

STUDIES ON THE ETIOLOGY OF TSUTSUGAMUSHI DISEASE.

The focal point of the investigation of tsutsugamushi disease lies in the study of its etiology. In 1893, Kitasato (78) reported that he found a small body in the red corpuscles of a patient with this malady and claimed that it was a plasmodium, but other investigators did not confirm this. Tanaka set forth an hypothesis of a protozoan parasite, but substituted a proteus for this in 1904, saying that he had identified a proteus at the site of the insect bite. Later, in 1906, he discarded this hypothesis and stated that the disease was due to the toxin of akamushi. Ogata and Ishiwara found a polymorphous substance, in 1905, in patients and cadavers, regarding it as a living, independent organism and calling it an ameboid protozoon. In 1906 they claimed that it was a sporozoon and stated that they were able to produce an immune serum by its cultivation. In 1910 (140) they determined its life cycle and named the microbe *"Gregarina tsutsugamushi."* Ogata believed, from the type of its life-cycle, that it was not a protozoon, but a schizomycete and, in 1912 he set forth a schizomycetic hypothesis for the etiology of tsutsugamushi, since then reporting on it frequently. Since 1906 Hayashi has been finding small, rod-shaped, spheroidal and ring-shaped bodies in and around the tissue-and blood-cells at the site of the akamushi bite and in the viscera of human and animal subjects, and claims them to be protozoa. Comparing them with *Theileria parva,* the causative agent of coast fever in cattle in East Africa, he names his protozoon *"Theileria tsutsugamushi,* sp. nov." He also describes its life cycle, but has been unable to cultivate it.

Since 1905, Kitashima and Miyajima (80 - 84) have been finding small spherical, or oval bodies of irregular size in the body of the akamushi parasitizing field rats and have studied it as the etiological agent. It is not only found in

akamushi but also in the body of their pupae and larvae; in the adult it was found in the ovary. Morphologically it might be a saccharomycete, but such yeasts are not found in trombicula or acarina, therefore they named it *"Chlamydomyces akamushi."* In 1917 they found granular bodies in the tissue of akamushi and microscopic granules on the surface of ordinary agar and ascitic agar cultures, which they claimed were akamushi bodies. Animal experiments with the cultures of these granules were said to have shown positive results and they were claimed as the causative agent; but later this claim was withdrawn. These akamushi bodies are, no doubt, saccharomyces; but their bearing on the etiology of tsutsugamushi disease is unknown and requires further study.

In 1915, (121) Nagayo, Miyagawa, Imamura, and Tamiya found small particles of irregular size and staining-property in the blood, lymphnodes and spleen of infected monkeys and claimed that they were the causative agent. In 1917 they succeeded in cultivating a bacterium on Löffler's serum-agar with the blood of an infected monkey and they were able to transplant it through three generations. These bacteria resembled saccharomyces in their arrangement, but when stained at the beginning of cultivation each bacterium was stainable in two ways. After subcultivation this peculiarity was lost, the bacteria staining in only one way. They claimed that this microbe belonged to an unknown species of bacteria. When cultures of it were injected into a monkey, it developed tsutsugamushi disease, but the agglutination test was negative.

In 1919, Ishiwara and Ogata, Jr. (59), found a very small, round organism, by means of special staining methods, in the lymphnodes of patients dying from tsutsugamushi disease and later it was found in the spleen, heart, and at the site of the insect bite. Moreover a similar small body was found in the tissues of monkeys that had contracted this disease. When the blood of a patient was placed in a liquid medium containing ascitic fluid, clouds of fine particles would be found at the bottom of the test-tube after two or three days of incubation. They found the same organisms in this precipitate and reported the fact in 1920. They were able to sub-

cultivate it through five generations. By injecting this culture subcutaneously and into the testes of monkeys, they produced swelling of these tissues and a rise in temperature, but they did not notice any leukopenia, and the immunity reaction was negative. They claimed that these small bodies had an important bearing upon the etiology of tsutsugamushi disease, but they did not clearly establish the genus to which they belong. In 1924, Nagayo and his co-workers found a "causative agent" in the blood and tissues, especially the skin, lymphnodes and spleen. This agent has various forms, some of them are short and rod-shaped, with deeply staining poles, or they resemble diplobacteria, and micrococci. These bodies very closely suggest the rickettsiae of typhus, or of Rocky Mountain spotted fever, but while they stain with alkaline Azure II, they do not take the Giemsa stain. Similar small bodies are found in the large cells, or histiocytes, and outside of them, as well as in the lymph and plasma. It is claimed that they are the causative agents of tsutsugamushi disease.

In 1923 Sellards (169) inoculated a monkey with tsutsugamushi disease virus supplied him by the writer and cultivated and described an organism recovered from its blood. He believes it to be related to the Rickettsiae. It is, however, strange that the inoculation of these cultures into his animal brings about hemorrhagic conditions never observed in our series of several thousand animal experiments. Furthermore, the results he obtained in the monkey were insufficiently striking to prove that he had isolated the true causative agent of tsutsugamushi disease.

According to our experience, the monkey is the only animal which can be used for the purpose of studying the etiology of this disease and to determine whether or not another monkey has contracted the disease. On the other hand, if there be neither typical fever curve nor leukopenia, and the immunity gives positive results, it is difficult to decide whether or not the animal has become infected; even the Japanese monkey shows a susceptibility to the disease that varies with the individual.

For this reason, I have drawn up rigid rules for the experiments with monkeys: I have considered as positively in-

fected animals only those that have a definite incubation period (6 - 13 days, avg. 8.6 days), followed by a typical fever-curve and marked leukopenia (one-half to one-third the normal white-count) and positive immunity tests. If either fever-curve or leukopenia were atypical, but the immunity test positive, the case was considered doubtful; those giving negative immunity tests with typical fever curve of leukopenia, or both, were considered negative cases. As the body temperature and blood-pictures are influenced by the weather and by the disposition of the animal, careful consideration must be given these factors in the experiments.

1. Morphological Investigation of the Causative Agent of Tsutsugamushi Disease.

A. Experimental Material and Methods.

Since it is an established fact that the causative agent of the disease is found in the blood, spleen, lymphnodes and bone marrow of patients or infected animals, we have used freshly-drawn blood from these, regional lymphnodes from patients, and various organs from infected animals, removed at different stages in the disease. These materials were examined in their natural state and then smears and sections were made for microscopic study.

To fresh blood or extracted tissue fluids, we added saline solution, or (to prevent coagulation) 1.5% sodium citrate, or hirudin, before the examination. This was undertaken either with ordinary transmitted daylight, or under the dark-field with artificial light. Furthermore, blood with the above percentage of sodium citrate was added and the mixture centrifugated in order to separate the cells and the plasma, after which each was examined separately. The layer of leukocytes was repeatedly centrifugated and the plasma removed. The fluid in the vesicles of the eruption was also examined.

For vital stains we used polychrome methylene blue, May-Grünwald, brilliant violet, Bismark brown, methylene blue, methylene purple, neutral red, picric acid and borax carmine, as well as the Giemsa stain as recommended by de Rocha-Lima (155). In preparing smears, blood, serum, or tissue fluids were smeared over glass slides or cover-glasses, or

thick smears were prepared on cover glasses by Ross' (156) method. According to the purpose of the examinations, these specimens were fixed either with ethyl or methyl alcohol, or by the flame, and some by sliver impregnation. For this the specimens were first dried either in air, or in the vapor of a mixture of acetic and osmic acids. Other fixing-methods were used according to the needs of the different stains.

As to staining methods; the Giemsa, Romanowsky, May-Grünwald, triacid, polychrome methylene blue, methylene blue, carbol fuchsin, and especially Manson's borax methylene blue and silver impregnations were used. Löffler's, Imai's and Hidaka's (50) methods for staining flagellates were likewise employed. For silver impregnations various percentages of silver nitrate were used, with a variable length of time in the incubator. We obtained satisfactory staining with all these methods.

The tissue specimens were fixed with methyl or ethyl alcohol, formalin, mercuric chloride-alcohol, and Zenker's fluid; they were then imbedded in paraffin and very thin sections were cut at one or two microns. These sections were stained by the methods noted and Levaditi's silver impregnation was resorted to repeatedly.

B. Results of the Experiments.

No special changes were seen in the hanging drop or capillary specimens of erythrocytes and leukocytes, but we found a small body in the plasma. This lies singly, or in chains which present the appearance of diplococci or bacilli, and seem to possess transparent capsules. Their size varies within the limits of 2 to 0.7 microns and they are actively motile, wheeling in a spiral manner, contracting and expanding, and moving among the blood cells or over their surfaces. When they appear in large numbers these bodies present a picture of ten or more diplococcoid bacteria in clumps. Beside these, large cocci, ovoid bodies with bud-like processes, or small branching forms like coral are occasionally seen. A large example of the last type measured 10 microns in length and one in width. These are more slowly moving than the others, they do not take any of the vital stains and are not stained in the fixed specimens. They do not stain with the various

methods ordinarily employed for staining protozoa or spirochaetae, remaining untinged by any method in fresh and fixed specimens.

These bodies are only occasionally found during the incubation period and the first stage of the disease, but appear suddenly in large numbers in the latter part of the second and third stages, being also found during convalescence. They were likewise observed in the blood of monkeys, appearing in that of two of these two weeks after injection. One of these monkeys harbored them during the third stage, but their number is far smaller in monkeys than in man. They were also observed in the urine that was removed under aseptic precautions from three patients, some of the specimens showing moderate or large numbers of them. Their structure and motility was the same as in the case of those noted in the blood.

Ogata found rod- or spindle-shaped, highly refractile bodies in the blood or necrotic material from the skin of patients with tsutsugamushi disease. These particles had a moderate degree of motility, wheeling and vibrating. He also found foamy bodies and claimed that they were sporozoa or ameboid protozoa which he was able to stain. Thus, what he found and what we found are not identical. Hayashi described spherical or worm-like, stainable bodies in the red corpuscles of patients. He called them piroplasma. The small bodies we found resemble those described by Hayashi morphologically, but as ours can not be stained, they are not the same.

Our small forms are, therefore, entirely different from those reported by other observers, but that they have no relation to the causative agent of tsutsugamushi disease is shown by the following facts. In the first place, such particles are occasionally found in small numbers in normal human blood; that of a man of 54, who had measles, contained them in large numbers. Consequently they can bear no relation to the causative agent. Furthermore, the urine of a patient who had shown the bodies in his blood was removed asceptically and many of them were noted in its sediment, after centrifugation and several washings. They were injected into monkeys, included in the sediment, but no fever nor leukopenia resulted,

and immunity tests were entirely negative. Thus we see that these bodies have nothing to do with the etiology of the disease and if this be so, what are they? It is very probable that they belong to what Schmidt (163) calls "Hämokonien;" they are an altered constituent of blood and are caused by febrile disease and metabolic disturbances.

When a fresh blood specimen was examined under the dark field, we noted spirochaetoid forms, varying greatly in their length and width. Thin ones are as large as *Treponema pallidum* and their width varies from too little to measure with a Leitz 2 mm. oil immersion up to one micron, while their length varies from one micron to several times the diameter of an erythrocyte. They are either straight, or have two or three slight, wavy curves. The pointed end terminates in a squared or rounded tip and their surface is not lustrous. They appear to be homogeneous and structureless. Their motion is not spiral, but merely undulating and can not be considered as constituting automatic progression. They appear rather to move with the flow of the fluid in the specimen. They are found freely distributed through the plasma, but occasionally attach themselves to red cells and to platelets. Their extremity has an active waving motion. Found constantly in the blood of patients with tsutsugamushi disease and in that of infected animals, they were also occasionally observed in that of normal individuals, patients with other diseases, and normal animals; the only difference being that they were found in far greater profusion in the blood of sufferers from tsutsugamushi disease than in that of the other cases. They were also observed in the fluid from vesicles, in the lymphnodes and tissue-fluids of animals but we could not stain them by any known methods of vital or fixed staining.

Consulting the literature, the small bodies observed by Chambers (20) in the blood of patients with exophthalmic goitre and other diseases, as well as in normal human beings, correspond to ours, except that they are capable of being stained with gentian violet. Chambers calls them spirochaetae. Miyaji (102) found similar structures while studying Kurloff's bodies in the blood of guinea pigs and notes that they are not stainable by any method; therefore they are

pseudospirochaetae and not true organisms. As Miyaji notes, these have a certain relation to the erythrocytes and platelets and are surely a degenerative product, very probably of an albuminoid or lipoid constitution.

Red and white corpuscles and platelets were very scrupulously examined in fresh specimens, but no small motile bodies could be found. When a fixed specimen is stained by Giemsa's method, small blue bodies will occasionally be seen in the red cells. One or two chromatoid granules were noted in these bodies, which were also present in the fixed specimens of regional lymphnodes and in the spleen of infected monkeys. Some of the red corpuscles have 8- or S-shaped rings, which are Cabot's rings. There are also slightly larger basophil granules in the red cells, but they are not specific for tsutsugamushi disease, constituting regenerative forms in an anemic condition and sometimes seen in other diseases.

The white blood corpuscles may often contain dot-, rod-, or ring-like granules which may be found in chains of two or three and are known as "Döhle's bodies." They may be found in the blood during the fastigium as well as in that of other infectious diseases such as measles and typhoid. We frequently found azure granules of rather large size in the large mononuclear cells, often forming pairs, but they have nothing to do with the etiological agent of tsutsugamushi disease. The blood-platelets contain nothing which calls for special remark.

We have seen various substances in and around the cells in smears from the regional lymphnodes and spleen. They are grouped in large or small masses and, when stained by Giemsa's method, they exhibit metachromasis. Some of the round, oval, spheroidal, and irregularly-staining substances contain dark red chromatin-like granules and resemble those noted by Hayashi and by Nagayo, but they can not be considered as organisms, judging by their regional relationships and staining properties. Occasionally hemoglobin granules may be observed.

In histological sections various methods were applied to preparations of lymphnodes, liver, spleen, and bone marrow, but no substances requiring special description were noted.

2. Cultural Experiments.

A. Experimental Material and Methods.

Former investigators have made repeated experiments in the cultivation of the etiological agent in tsutsugamushi disease, the majority obtaining negative results, which indicates the difficulty encountered in cultivating it. We have tried every cultural method devised in our study of the virus. We repeatedly employed Löffler's serum medium, which was recommended by Nagayo, but all our attempts failed to produce a growth. Nicolle's simplified blood-agar medium was tried, blood being drawn from normal human beings, rabbits, or monkeys and used after defibrination at 45° C. We also used the spirochaete culture method of Schereschewsky. The Noguchi method for growing avian spirochaetes was also tried, using ascitic or hydrocele fluid with fresh aseptic bits of rabbits' heart and kidney. Again, we used horse or rabbit blood diluted four times with sterile water. In the case of all these media cultures were repeated under aerobic, partial tension, or strictly anaerobic conditions and control experiments were carried out in each instance. The cultures were incubated either at 37° or 25° and examined at intervals during the following days, microscopically if needs be. Some of them were left in the incubator for one to three weeks for observation.

The materials used for inoculating the culture media consisted mainly of blood obtained from the basilic vein of patients during the febrile stages of the disease and diluted with 1.5% sodium citrate. Lymphnodes removed from patients, or the spleen and bone marrow from infected monkeys, were cut into small pieces, or emulsified, and placed in the media. Sometimes citrated blood was placed in the incubator and observed for several days.

As mentioned in the preceding section, we used the dark field apparatus with the hanging drop, or capillary method, or smears were stained and carefully examined under the microscope.

B. Results of Cultural Experiments.

In the Schereschewsky medium there was a filamentous turbidity along the inoculation-streak in coagulated horse blood. With Noguchi's ascitic, or hydrocele fluid media, diluted rabbit's blood, or the horse-blood medium there was a clear, white, ring-shaped zone around the piece of tissue suspended in the bottom of the tube, or in the case of the blood a dark red turbid ring was formed near the bottom, but these were also observed in control tubes.

Microscopically, the filamentous turbidity in the Schereschewsky medium was found to be crystallized albumin in various forms, and had none of the specific properties of bacteria. In Noguchi's media an examination of the clear zone showed that there were a few spherical, or irregularly-shaped particles showing Brownian motion and most numerous in the turbid area. Most of them were lustreless. Pseudospirochaetae were found among them, some of which might be attached to well preserved erythrocytes for several days. The small bodies do not take vital stains and are not stained in fixed specimens. No small bodies were found in the red or white blood corpuscles at the bottom of the tubes. The examination of the serum on the surface of blood agar showed the same particles and pseudospirochaetae that were observed in the other media; the blood corpuscles were well preserved. Staining reactions were quite negative in these cases and no small bodies were seen in the erythrocytes.

3. Biological Studies of the Causative Agent.

The biological study of a causative agent of an unknown nature is very important, therefore I have investigated that of tsutsugamushi disease from various angles.

It is certain that this agent is contained in the blood of patients and infected animals, but we do not yet know when it appears or disappears. Therefore we drew blood from two patients in the incubation period, when they merely had tender lymphnodes (although they developed the typical disease later) and injected this blood into monkeys. After twelve- and seven-day periods of incubation, respectively, monkeys Nos. 20 and 60 developed typical attacks of tsutsugamushi disease. Experiments were carried out in three in-

stances to determine the date of disappearance of the virus from the blood. In the first, blood was taken on the eighteenth day of the disease, two days before convalescence began, and was injected into a monkey, which came down with the typical onset and disease after an incubation period of fourteen days. In the second, a very severe case, blood was taken on the day convalescence began and was injected into a monkey which developed a typical attack of the disease after an eleven-day incubation period. In the third case, also very severe, the blood was drawn twenty-two days after the beginning of convalescence and was injected into a monkey, which failed to develop any symptoms whatsoever. (Fig. 21, 22).

From this it will be seen that the causative agent of the disease is already present in the blood during the incubation period, remaining there during the entire period of fever. When convalescence begins the virulence of the virus appears to diminish, which is due, of course, to the development of an immunity. The virus disappears in a comparatively short time after the beginning of convalescence.

The causative agent exists in the regional lymphnodes and in large amounts in the tender lymphnodes during the incubation period. I removed one of these nodes during that period from Case No. 1, injecting it into monkey No. 71, which developed a typical attack after six days of incubation. As proof that the regional nodes contain the causative agent, one of these was removed after the onset of fever and 1 gm. of it was emulsified and injected into monkey No. 61, which also developed a typical attack after nine days of incubation. On the other hand, in two cases that had atypical cases the lymphnodes removed in the early stages did not produce the disease in monkeys.

The foregoing experiments show that the tender lymphnodes of patients who have the disease in a typical form contain the causative agent, which can be transmitted to monkeys by injection of emulsions of these nodes; the virus is contained in them not only during the febrile, but also during the incubation period. Thus, the causative agent entering through the bite of akamushi, passes through the lymphnodes during the incubation period and diffuses into the bloodstream.

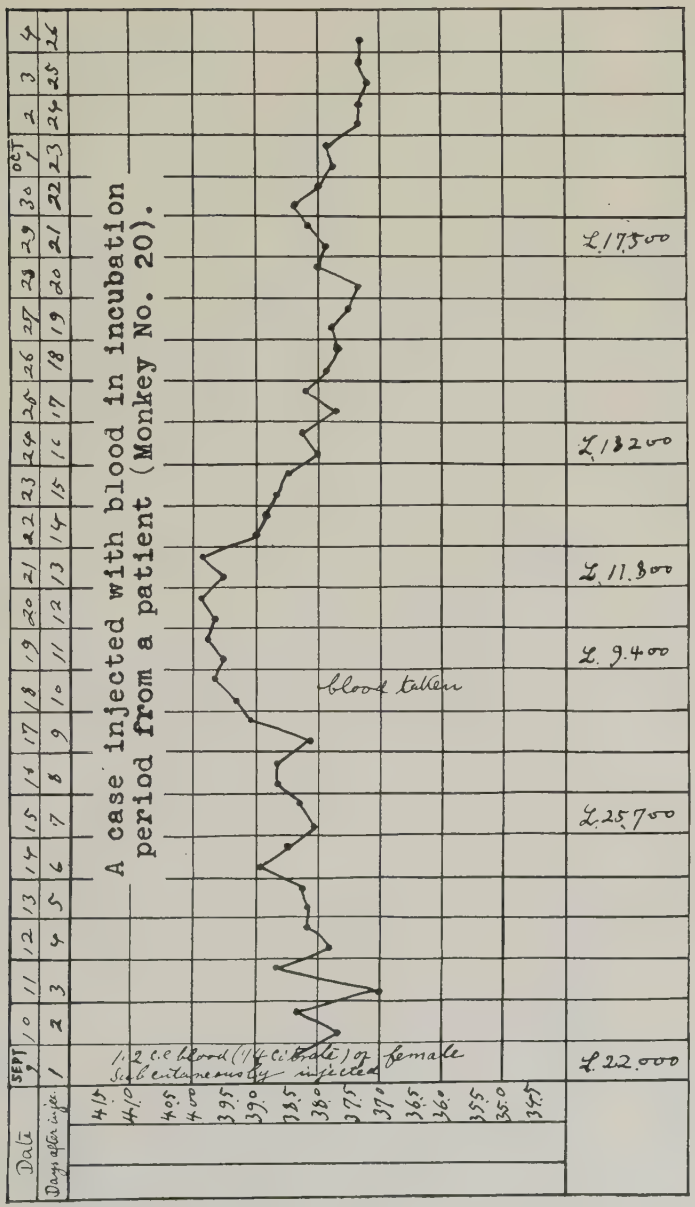

A case injected with blood in incubation period from a patient (Monkey No. 20).
1.2 c.c. blood (1/4 c.c. date) of female Sub cutaneously injected
blood taken
£17500
£13200
£11.300
£9.400
£25700
£22.000

Figure 21

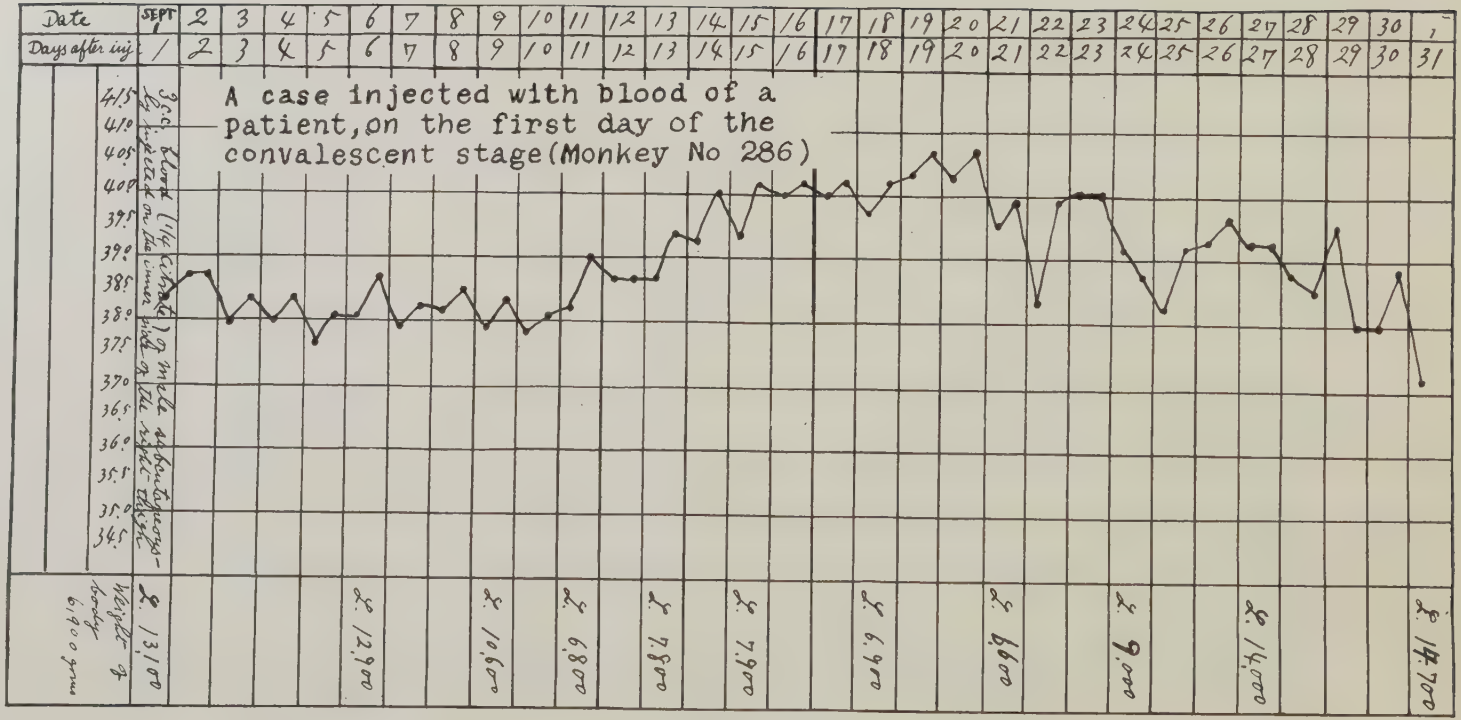

Date
Days after inj.
A case injected with blood of a patient, on the first day of the convalescent stage (Monkey No 286)
3 c.c. blood (1/4 citrate) injected on the inner side of the right thigh
Weight of body 6,900 grms.

Figure 22

In order to determine the amount of blood required to produce a "take" in monkeys, the following experiments were performed.

Two monkeys were inoculated with 0.001 c. c. of blood from a severe case and a like amount from an infected monkey. One of these (No. 85) developed fever after thirteen days of incubation, but the course thereof was not typical. There was leukopenia, but an immunity test gave negative results. The other monkey (No. 214) had no fever. It was reinoculated, this time with 0.01 c. c. of blood and, after an incubation period of thirteen days, it developed a fever (Fig. 23). Monkey No. 281 received 0.005 c. c. of blood without developing any fever, while the immunity test was positive. Monkey 213 received 0.01 c. c. and fever came on after eleven days' incubation.

Thus, the amount of blood required to cause the disease in monkeys is 0.01 c. c., while 0.001 c. c. may sometimes suffice. The strain of virus used in this experiment can produce symptoms after an incubation period of eight or nine days, therefore the incubation period is prolonged after the inoculation of as much as 0.01 c. c. of blood, while there is no prolongation if 0.1 c. c. be injected.

In order to ascertain which element in the blood contains the causative agent of the disease, we carried out the following experiments. The blood was diluted with sodium citrate solution to prevent coagulation, the plasma was separated from the cellular elements by centrifugation, or the blood was left in an ice-chest to separate spontaneously by gravity. Each element thus separated was then injected into monkeys, the cells being washed several times before injection with normal saline solution.

Out of nine monkeys injected with plasma, six remained absolutely negative, two were doubtful, and one died of intercurrent infection. Therefore there was none, in this series, that gave a frankly positive result. Blood filtered through the Berkefeld filter No. V, was injected into four macaques, two of which died and two remained negative. These results agree with those obtained by Miyajima, Kitashima, and Nagayo. According to Miyajima, the causative agent does not pass a porcelain filter. The serum obtained after clotting gave

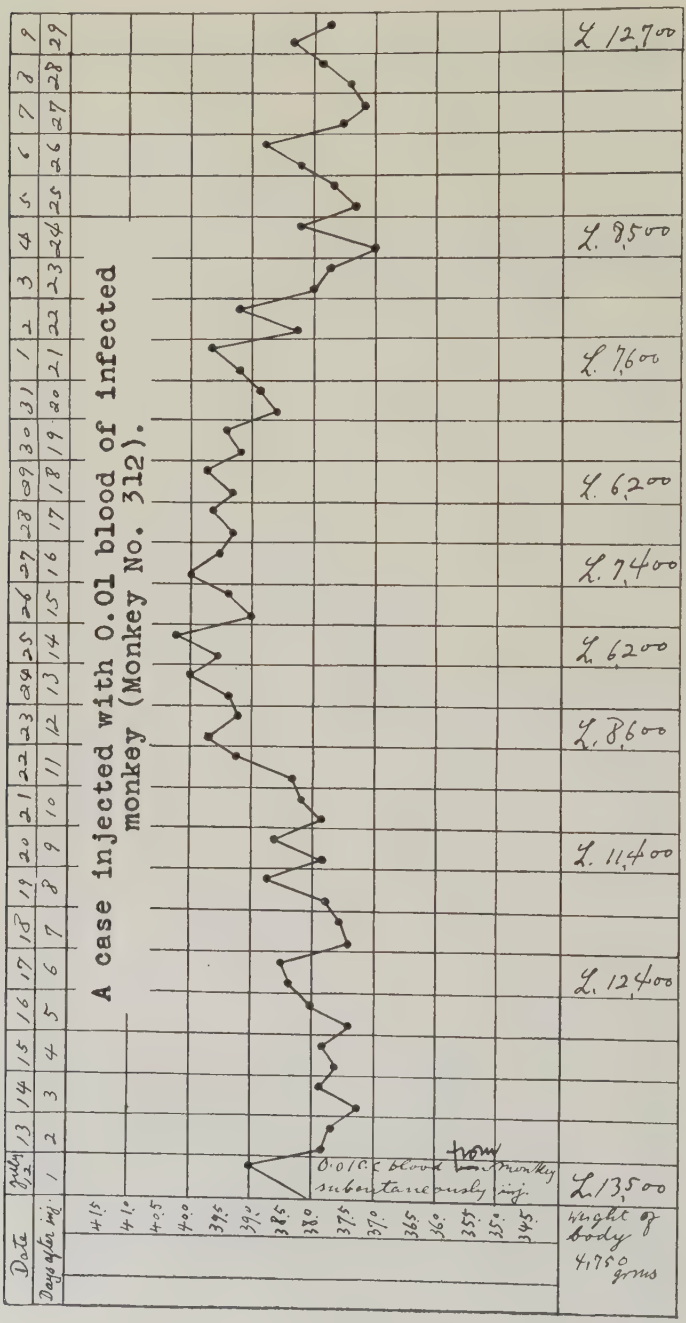

A case injected with 0.01 blood of Infected monkey (Monkey No. 312).
Date
July 13
Days after inj. 1
0.01 c.c. blood from monkey subcutaneously inj.
Weight of body 4,750 grms
¥ 13,500
¥ 12,400
¥ 11,400
¥ 8,600
¥ 6,200
¥ 7,400
¥ 6,200
¥ 7,600
¥ 8,500
¥ 12,700

Figure 23

negative results when injected into monkeys. On the other hand, seven monkeys injected with washed blood corpuscles all gave positive results. (Figs. 24, 25, 26).

This indicates that the causative agent has a close relation to the formed elements of the blood or has the same specific gravity as theirs and that it is of a form that passes with difficulty through the pores of a filter. If this agent were merely suspended in the plasma, centrifugation for half an hour at 2000 - 3000 r.p.m. would not completely remove it from the supernatant fluid. Again, there is no reason why this supernatant fluid separated by the mere force of gravity in the ice-chest should be innocuous. The causative agent must, therefore, have some unknown physico-chemical properties. I have washed the blood cells ten or more times with citrate solution, yet they remained infective; therefore I suspect, after repeatedly studying these phenomena, that the causative agent has a very close relationship to the corpuscles. If these contain the noxious element it must be attached either to the red or white cells, or to the platelets, or some combination of all these.

A. Red Corpuscles.

The changes in the erythrocytes are indistinct. There is merely a slight decrease in their number and hemoglobin content as would be seen in any acute febrile malady. Morphologically there is more or less poikilocytosis and increase in basophil granules, but there are no small stainable bodies or granules worthy of special examination. It is difficult completely to separate erythrocytes from the leukocytes and platelets, for a few of these will always remain mixed with them; therefore it is difficult to obtain pure experimental results with separated erythrocytes. In 1917 we injected two monkeys (Nos. 36 and 38), one of them with a mixture consisting chiefly of leukocytes and platelets and the other with material composed of erythrocytes. In the latter monkey the incubation period was greatly lengthened and fever did not come on till twelve days after inoculation; in the former, the incubation stage was only six days. This, and other morphological studies of the erythrocytes, indicate that they may have very little bearing on the etiology of the disease.

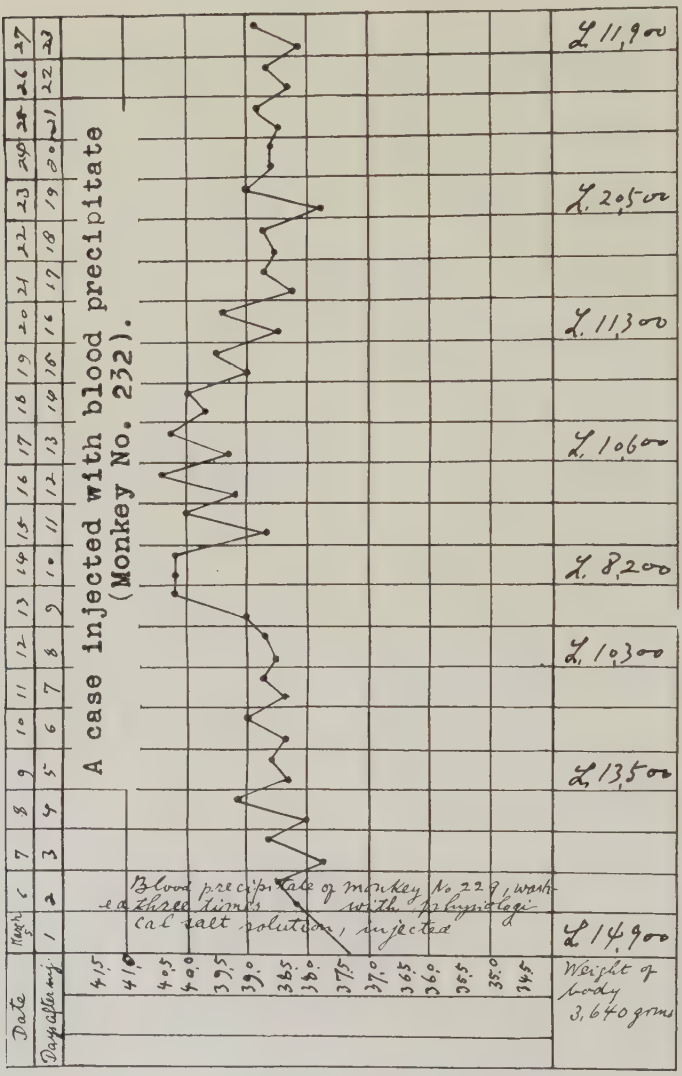

A case injected with blood precipitate
(Monkey No. 232).
Blood precipitate of monkey No. 229, washed three times with physiological salt solution, injected
Date
Days after inj.
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
Weight of body 3,640 grms
¥ 14,900
¥ 13,500
¥ 10,300
¥ 8,200
¥ 10,600
¥ 11,300
¥ 20,500
¥ 11,900

Figure 24

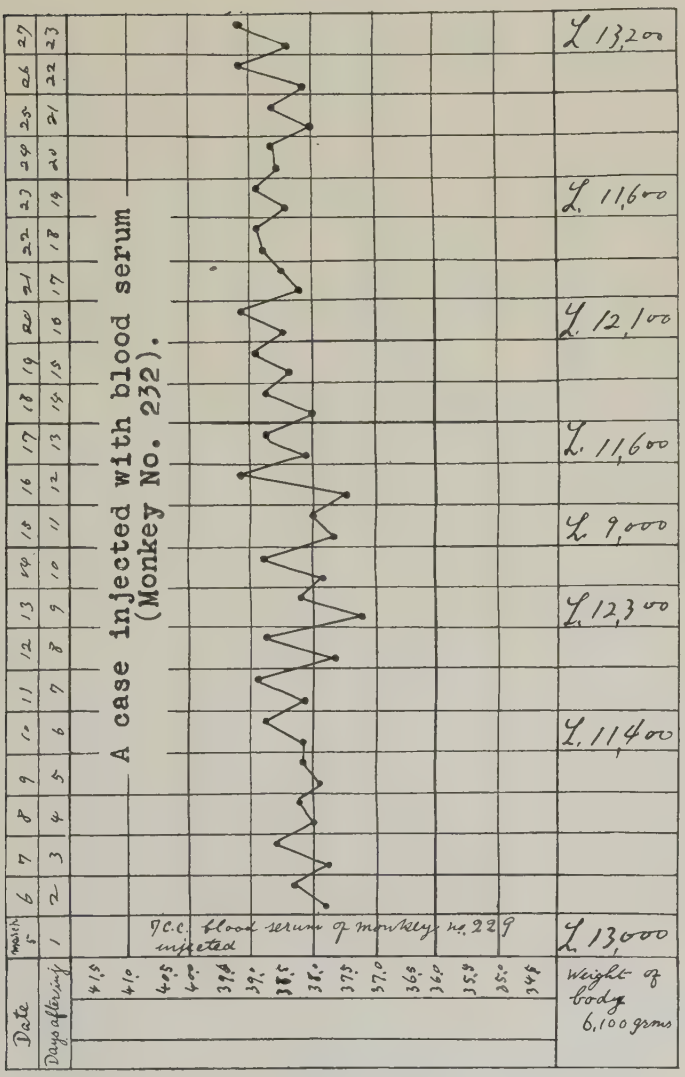

A case injected with blood serum
(Monkey No. 232).
Date
Days after injection
March 5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
7 C.C. blood serum of monkey no. 229 injected
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
L. 13,000
L. 11,400
L. 12,300
L. 9,000
L. 11,600
L. 12,100
L. 11,600
L. 13,200
Weight of body 6,100 grms.

Figure 25

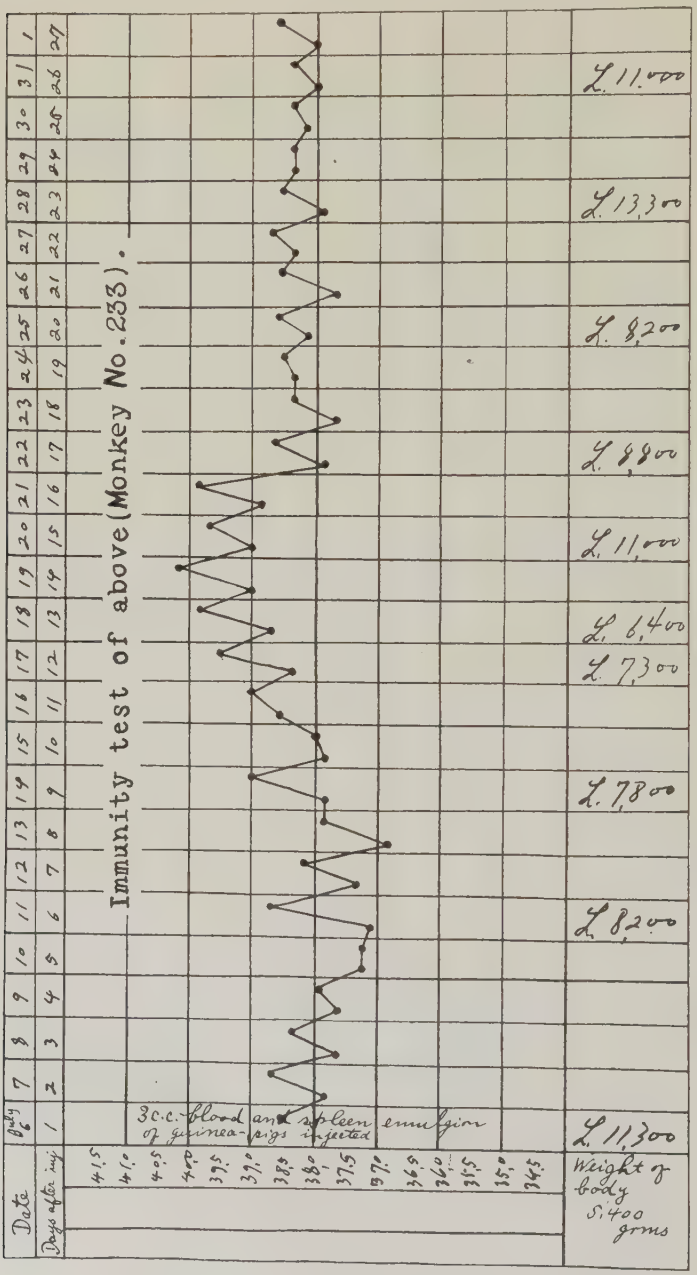

Immunity test of above (Monkey No. 233).
Date
Days after inj.
Body
Weight
30 c.c. blood and spleen emulsion of guinea-pigs injected
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
Weight of body 5,400 grms
¥ 11,300
¥ 8,200
¥ 7,800
¥ 7,300
¥ 6,400
¥ 8,800
¥ 11,000
¥ 8,200
¥ 13,300
¥ 11,000

Figure 26

B. Leukocytes.

We used two methods for collecting leukocytes: one was to precipitate them by centrifugation and the other to obtain them from artificially produced ascitic fluid.

a. First Experimental Method—Separation of Leukocytes from the Blood.

The experiments carried out in 1918 with the buffy coat (which also contained a few platelets and erythrocytes) and the red layer (containing also a few white cells and platelets) both gave positive results. But the incubation period after injecting leukocytes was very much shorter than the other. As the buffy coat did not consist of leukocytes alone, the results were indefinite and consequently, we selected the second method.

b. Second Experimental Method—Collection of Leukocytes from the Fluid of Ascites Artificially Produced.

A monkey, in the fastigium of an attack, was given from 300 - 500 c. c. of physiological saline solution intraperitoneally and after six and twelve hours this was aseptically withdrawn, any admixture of blood being strenuously avoided. Citrate solution was then added to prevent clotting and the mixture centrifugated to separate the formed elements from the fluid.

i. First Experiment (1917)

Monkeys Nos. 94 and 103 were injected with the centrifugate of this ascitic fluid, both developing fever after thirteen and fourteen days of incubation respectively. The precipitate consisted largely of white cells but very occasionally one erythrocyte might be found in several microscopic fields. The supernatant fluid was injected into monkeys Nos. 86 and 104. One developed fever in nine days, the other remaining negative.

ii. Second Experiment (1922)

The strain we used in this experiment was the "Meguro," originally obtained from an extremely severe case; it produced typical symptoms in monkeys after eight or nine days incubation period and had been passed through monkeys and guinea pigs for fifty-three generations. Monkey No. 237 had a fever lasting eleven days, maximum temperature 40.2°.. Five days after the onset of fever, at 9 P. M., 300 c. c. of salt solution was injected intraperitoneally and about 120 c. c. of the fluid was recovered twelve hours later. It was free from erythrocytes. The number of leukocytes per c. mm. was 1,900, that in the blood was 6,800 on the same day. As a control blood was drawn from the monkey at 4 P. M. and injected into another monkey.

iii. Main Experiment

This experiment was divided into three parts: one in which whole ascitic fluid was injected, one supernatant ascitic fluid, and the third the sediment of this.

Control Monkey: (No. 238) M., Wt. 4,800 gm.

1 c. c. of blood was taken from the right femoral vein of monkey No. 237 and, after being mixed with 0.25 c. c. of citrate solution, was injected into the mesial aspect of the right thigh of monkey No. 238. After ten days of incubation the animal had a typical attack of fever lasting ten days.

PART ONE.

(1) Whole Ascitic Fluid.

Monkey No. 242, F., Wt. 4,200 gms.

Whole ascitic fluid was injected into the right thigh of this animal in an amount of 5.3 c. c. (one-quarter citrate solution). This would contain a number of leukocytes corresponding to those injected into the control animal. The monkey had its onset of fever after ten days of incubation and it lasted nine days, with typical leukopenia and positive immunity test.

PART TWO.

(2) Supernatant Fluid of Ascitic Fluid.

Monkey No. 243, M., Wt. 3,000 gms.

The ascitic fluid obtained, as already described and mixed with one-quarter citrate solution, was centrifugated for half-an-hour and 8 c. c. of the supernatant fluid was injected into this monkey. This fluid contained no cells when examined under the dark-field. The monkey developed neither rise in temperature nor leukopenia and the immunity test was negative.

PART THREE.

(3) Ascitic Fluid Sediment.

Monkey No. 344, M., Wt. 6,100 gms.

10.5 c. c. of ascitic fluid (one-quarter citrate solution) was centrifugated for thirty minutes and the precipitate thus obtained was injected into the monkey. This precipitate contained no erythrocytes or blood platelets, consisting only of leukocytes. The monkey developed fever eleven days after injection and it lasted for seven days, with typical leukopenia and a positive immunity test.

iv. Third Experiment (1922)

A monkey (No. 247) injected with the Meguro strain was utilized for the collection of leukocytes. It had its onset of fever nine days after the injection and lasting nine days, the maximum temperature being 40.8°. At 5 P. M. on the fourth day after the onset, 400 c. c. of physiological salt solution was injected intraperitoneally and withdrawn aseptically the following morning. The number of leukocytes in this fluid was 5,300 per c. mm., that in the blood, 8,900.

Monkey No. 284, M., Wt. 3,770 gms.

We injected 5 c. c. of the whole ascitic fluid (one-quarter citrated) subcutaneously into the mesial aspect of the right thigh. The monkey developed fever eight days later, the highest temperature being 40.5°, and it lasted for ten days, with very marked leukopenia.

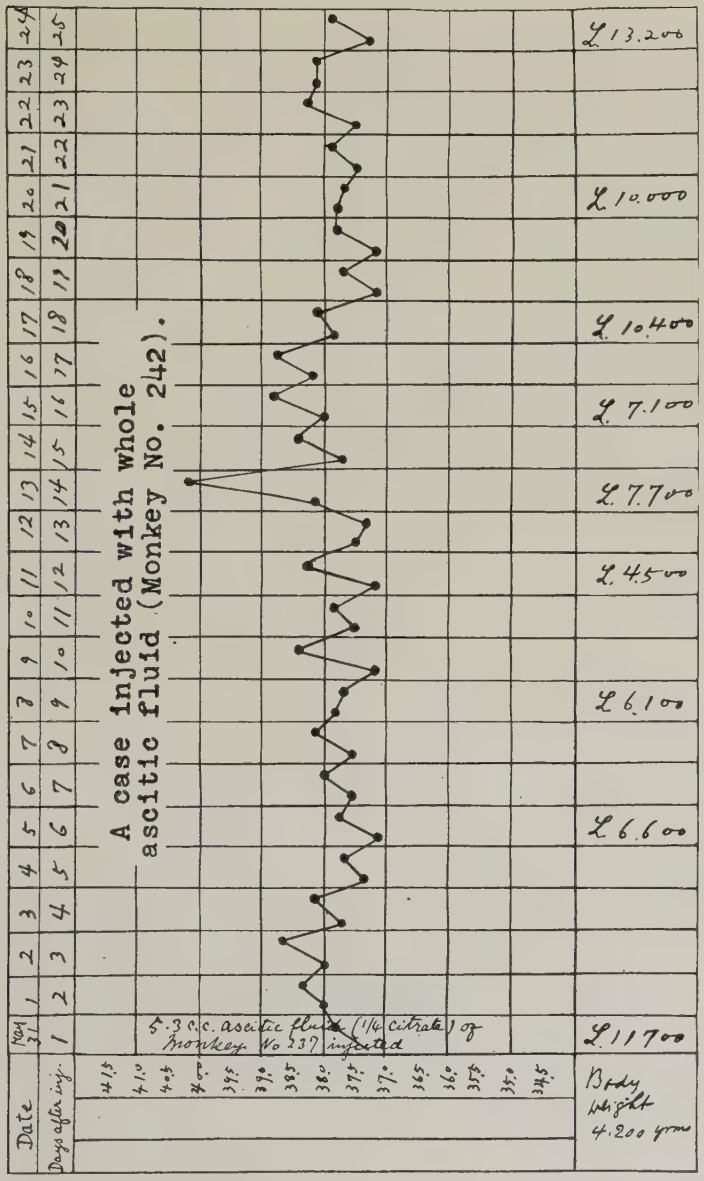

A case injected with whole ascitic fluid (Monkey No. 242).
5.3 c.c. ascitic fluid (1/4 citrate) of Monkey No 237 injected
Date
Days after inj.
May 31
Body Weight 4.200 grm
£13.200
£10.000
£10.400
£7.100
£7.700
£4.500
£6.100
£6.600
£11700

Figure 27

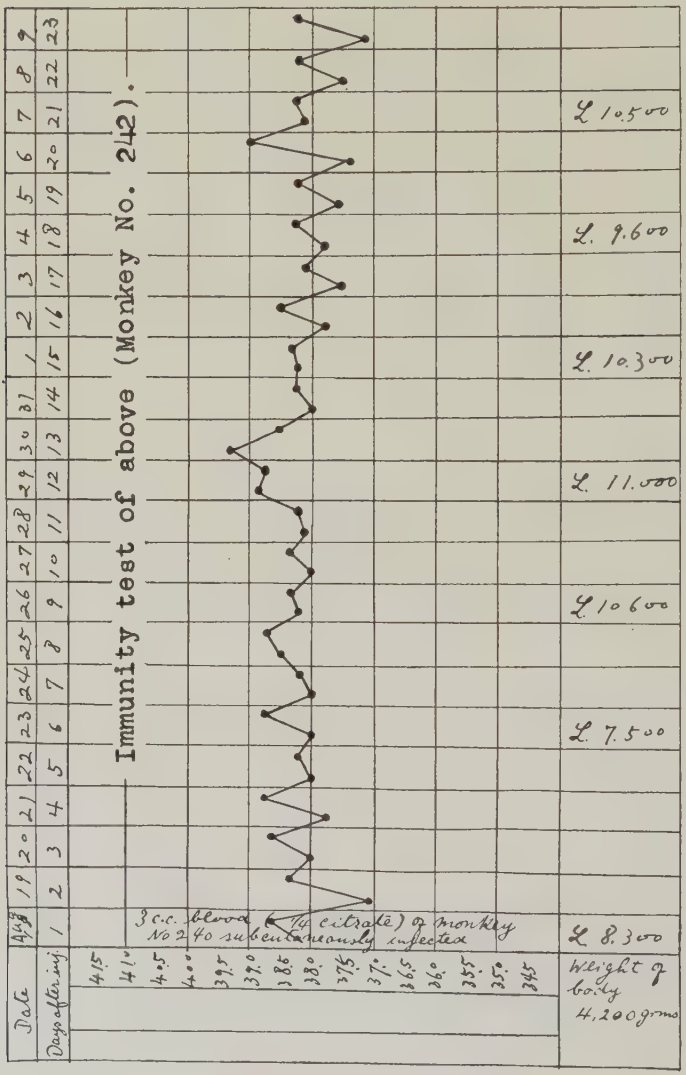

Immunity test of above (Monkey No. 242).
Date
Days after inj
4.15
4.10
4.05
4.00
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
3 c.c. blood (by citrate) of monkey No 240 subcutaneously injected
Weight of body 4,200 grms
£ 8.300
£ 7.500
£ 10.600
£ 11.000
£ 10.300
£ 9.600
£ 10.500

Figure 28

These experiments prove that the ascitic fluid produced artificially in infected monkeys contains the causative agent. When the whole ascitic fluid was injected into two monkeys (Nos. 242 and 248), both developed fever after eight or nine days' incubation, as did the control. When the sediment was injected, three monkeys (Nos. 94, 103, and 244) had typical onset of fever after a somewhat prolonged incubation period of eleven to fourteen days. (Fig. 29, 30, 31, 32, 33). On the other hand, when the supernatant fluid was injected, two of the three monkeys (Nos. 86, 104, 243) remained unaffected and their immunity tests were negative; the third developed symptoms after an incubation period of nine days.

The cellular elements of the centrifugate consist chiefly of polymorphonuclears and their fragments, with an admixture of small numbers of mononuclear cells, which are either lymphocytes or endothelial cells. Monkeys Nos. 94 and 103 received very few red cells. If the causative agent have any relation to the cellular elements, it must be with the polymorphonuclears. Monkeys Nos. 242 and 248 received about the same number of leukocytes as did the control and they both had an equally prompt and definite onset of symptoms. This indicates that the causative agent is in close relation to the polymorphonuclear leukocytes.

The monkeys injected with the precipitate had a prolonged incubation period, which may be due to the fact that it had been washed with salt solution and such manipulation might harm the causative agent. Two out of the three monkeys which were injected with supernatant fluid gave negative results, which agrees with those obtained with plasma and serum, but one gave a positive "take." This fact indicates that the causative agent was suspended in the fluid. It is very probable in this case that the causative agent had been liberated by the destruction of leukocytes.

C. Experiments with Blood Platelets.

The third group of formed elements in the blood is that of the platelets, the toxicity of which we examined on two occasions.

Monkey No. 329 had been injected with the "Meguro" strain and had fever after an incubation period of nine days,

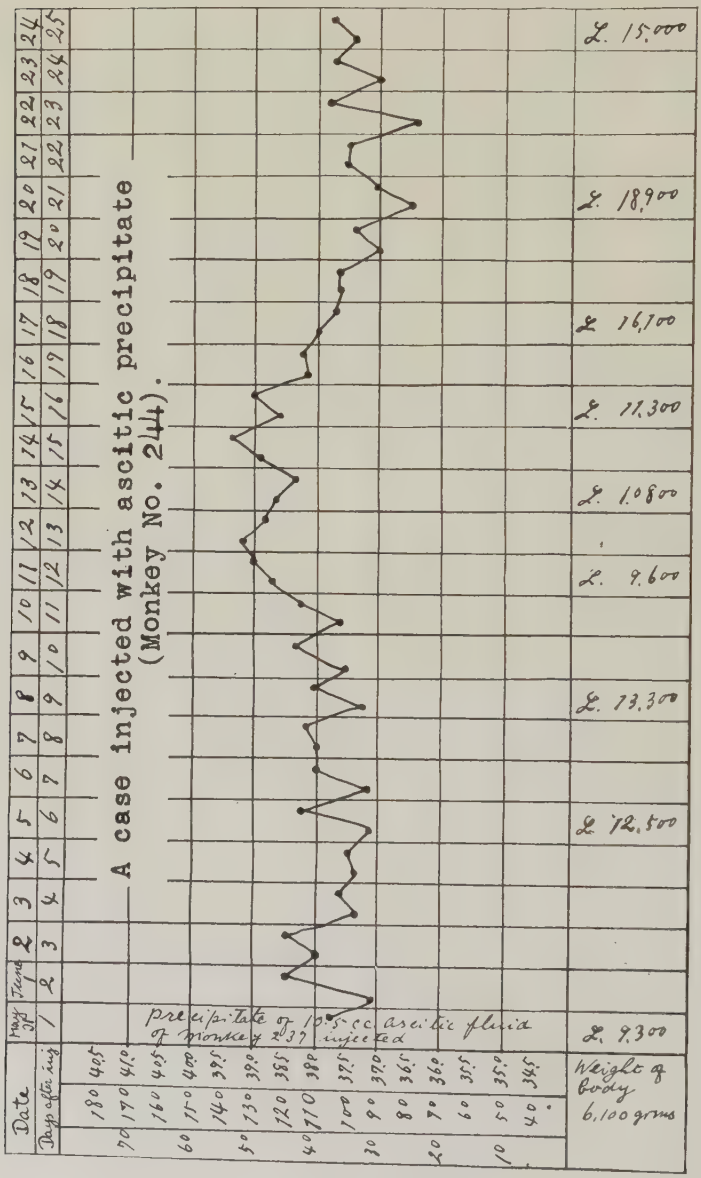

A case injected with ascitic precipitate
(Monkey No. 244).
precipitate of 10.5 cc ascitic fluid of monkey 237 injected
Date
Days after inj.
Temp.
Weight of body
6,100 grms
L. 9,300
L. 12,500
L. 13,300
L. 9,600
L. 10,800
L. 11,300
L. 16,100
L. 18,900
L. 15,000

Figure 29

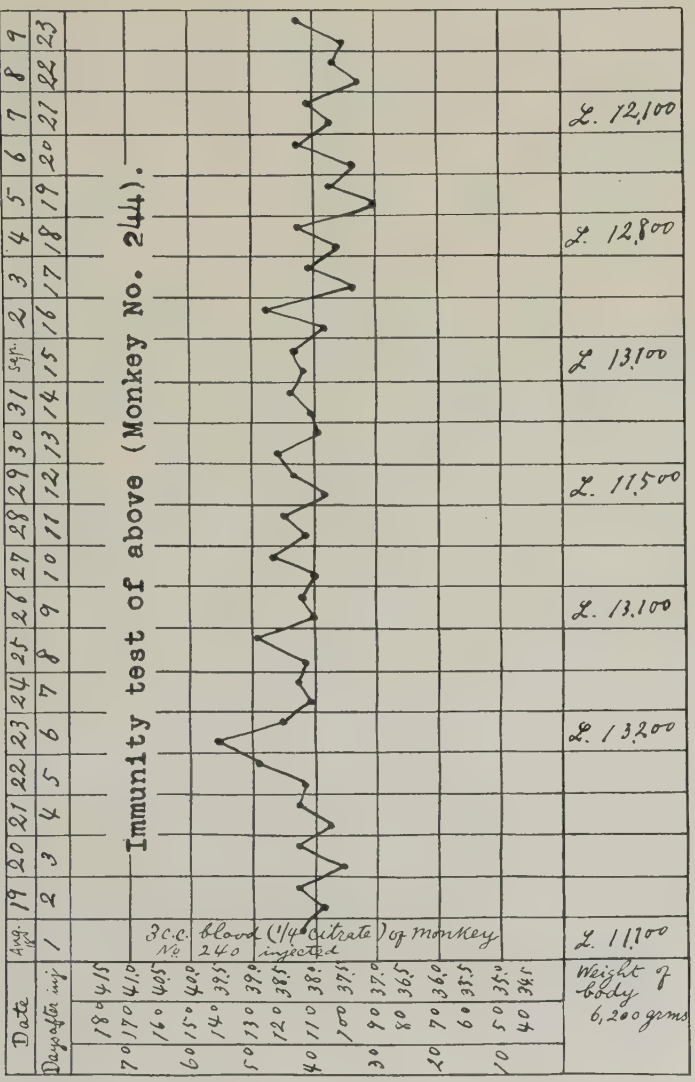

Immunity test of above (Monkey No. 244).
3 c.c. blood (1/4% citrate) of monkey
No 240 injected
L. 12,100
L. 12,800
L. 13,100
L. 11,500
L. 13,100
L. 13,200
L. 11,100
Weight of body 6,200 grms
Date
Days after inj
Avg. temp.

Figure 30

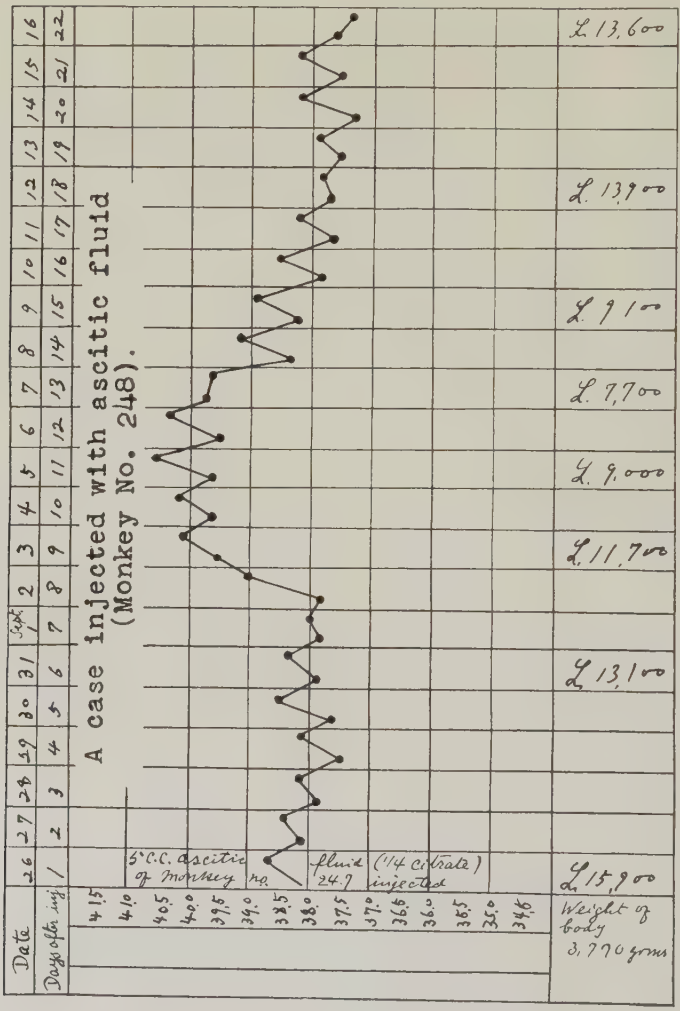

A case injected with ascitic fluid
(Monkey No. 248).
Date
Days after inj.
Sept.
26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.6
36.0
35.5
35.0
34.6
5 C.C. ascitic fluid of Monkey No. 247 injected
(1/4 citrate)
£ 13,600
£ 13,900
£ 9100
£ 7,700
£ 9,000
£ 11,700
£ 13,100
£ 15,900
Weight of body
3,770 grms

Figure 31

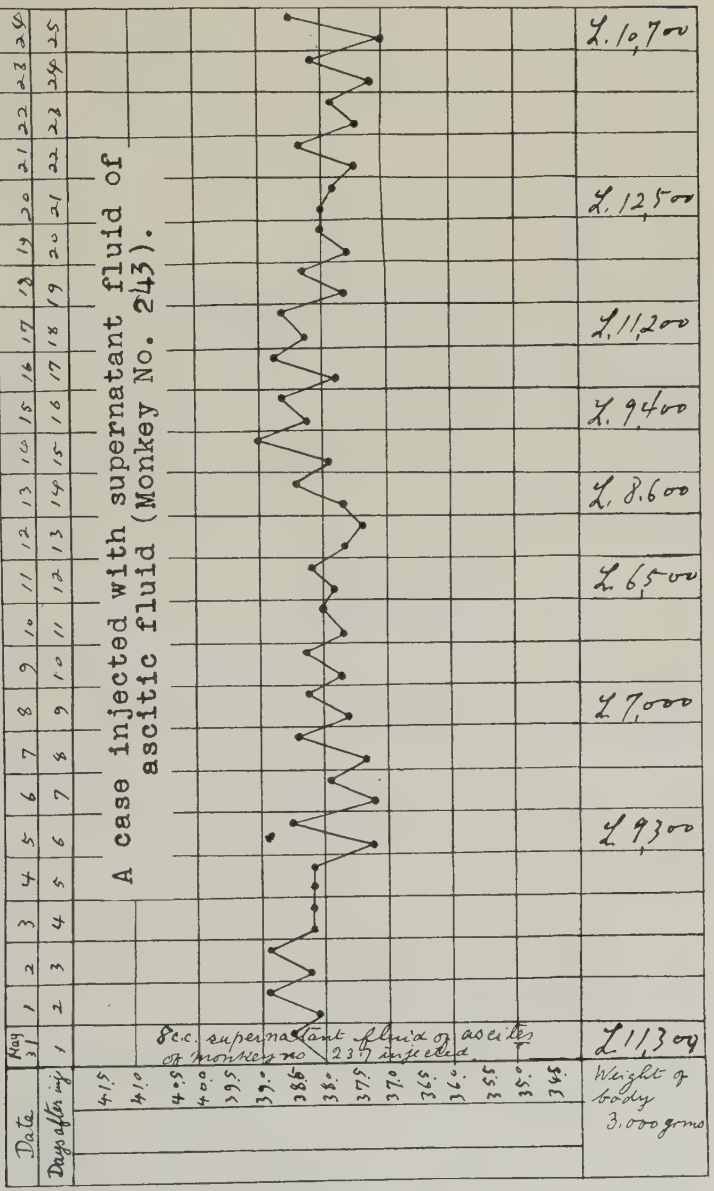

A case injected with supernatant fluid of ascitic fluid (Monkey No. 243).
8 c.c. supernatant fluid of ascites of monkey no 237 injected
Date
Nov 31
Days after inj
Weight of body 3,000 grms
£ 11,300
£ 9300
£ 7,000
£ 6,500
£ 8,600
£ 9,400
£ 11,200
£ 12,500
£ 10,700

Figure 32

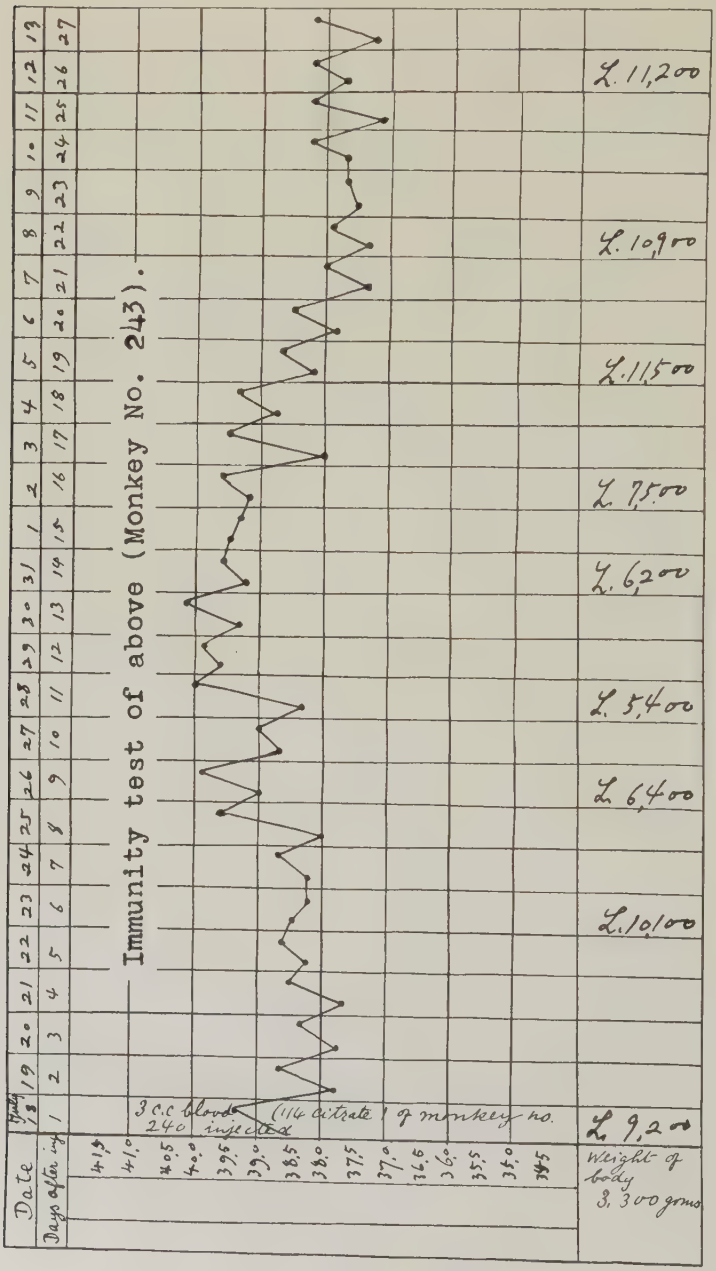

Immunity test of above (Monkey No. 243).
Date
Days after inj.
3 c.c blood + 1/14 citrate of monkey no. 240 injected
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.6
36.0
35.5
35.0
34.5
Weight of body 3,300 grms
L. 9,200
L. 10,100
L. 6,400
L. 5,400
L. 6,200
L. 7,500
L. 11,500
L. 10,900
L. 11,200

Figure 33

this lasting for nine days, the highest temperature being 39.1°. The animal was bled on the fifth day after onset and its blood was mixed with one-quarter citrate solution containing 1% glucose. The mixture was centrifugated for thirty minutes and the supernatant fluid removed. The precipitate was then washed three times. The supernatant fluid contained blood platelets after each washing, as determined with the dark field. These fluids were collected and centrifugated again for one-and-a-half hours. A smear of the precipitate thus obtained showed only blood-platelets. Both supernatant fluid and precipitate were used for injections.

The second lot of material was obtained from monkey No. 237, inoculated with "Meguro" strain and developing fever after an incubation of eight days. Fever lasted nineteen days, the highest temperature being 40.2°. The blood was mixed with the glucose-citrate solution and 5 c. c. of the mixture was placed in an ice-box for 20 hrs. after which the turbid layer was carefully removed and found to contain platelets only.

a. First Experiment.

i. Control Experiment.

Monkey No. 230, F., Wt. 4,800 gms.

We injected two c. c. of blood containing glucose-citrate, after eight days' incubation the animal developed a typical attack of fever lasting ten days, the maximum being 40.2°.

ii. Experiment with the Cellular Elements of the Blood.

Monkey No. 232, M., Wt. 3,640 gms.

The blood of monkey No. 229 (one-quarter diluted with the glucose-citrate solution) was centrifugated and the precipitate washed three times with this solution after which 3 c. c. of the cellular elements was injected. The monkey developed fever nine days later, which lasted eight days and reached a maximum of 40.4°.

iii. Experiment with Blood Platelet Precipitate.

Monkey No. 231, F., Wt. 3,300 gms. (Fig. 34).

The supernatant fluids obtained in experiment ii, were collected, centrifugated for one-and-a-half hours and 3 c. c. of the precipitate injected. There was no resulting change in temperature nor in the number of leukocytes, and the immunity test was negative.

iv. Experiment with Supernatant Fluid.

Monkey No. 233, M., Wt. 6,100 gms.

The supernatant fluid of experiment iii was injected, 7 c. c. being used. There was no change in the animal and a negative immunity test was obtained.

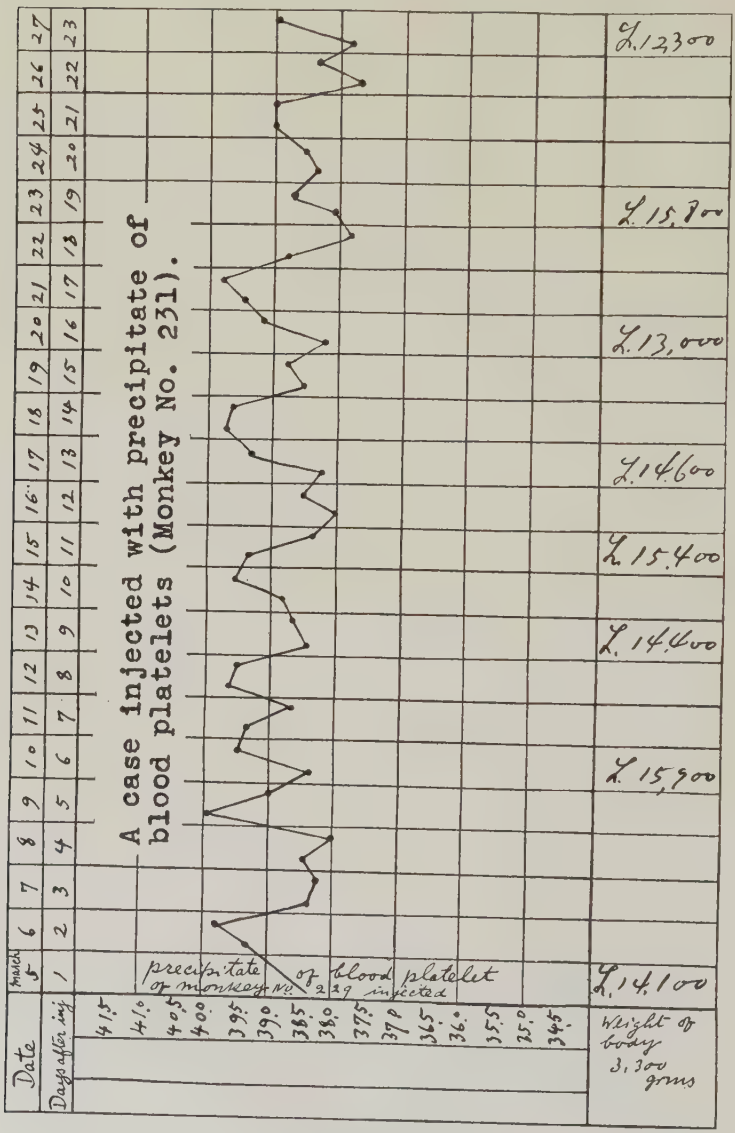

A case injected with precipitate of blood platelets (Monkey No. 231).
precipitate of monkey No. 229 blood platelet injected
Date
March
Days after inj.
Weight of body 3,300 grms
¥.14100
¥.15,900
¥.14600
¥.15,400
¥.13,000
¥.15,700
¥.12300

Figure 34

b. Second Experiment.

i. Experiment with Blood Platelets (Fig. 35).

Monkey No. 239, M., Wt. 3,500 gms.

The blood of monkey No. 237, containing glucose-citrate, was placed in the ice-chest for 20 hours, and the upper blood-platelet layer removed and injected. The animal showed no change whatsoever, except for a slight rise in temperature, the leukocytes were normal and the immunity test negative.

ii. Experiment with Precipitated Blood Platelets.

Monkey No. 241, M., Wt. 4,200 gms. (Figs. 36 and 37).

Three c. c. of the blood-platelet layer from experiment i. was centrifugated for three-and-a-half hours and the precipitate thus obtained was injected into this monkey without any change in the animal and with a negative immunity test.

iii. Experiment with the Supernatant Fluid of the Layer of Blood Platelets.

Monkey No. 240, M., Wt. 7,500 gms.

The supernatant fluid obtained from experiment ii., was injected with entirely negative results and a negative immunity test.

D. Summary.

The foregoing series of experiments show that the blood platelets have no relation to the causative agent in tsutsugamushi disease. As for the erythrocytes, it is difficult to separate them entirely from the other elements. Nicolle (129) claims to have shown that the causative agent of typhus fever is contained in the layer of leukocytes and we, too, have found that injections of this layer produce severer symptoms of tsutsugamushi disease with a shorter incubation period than does the layer of erythrocytes. Thus, it is indicated that the causative agent of tsutsugamushi disease has a close relationship to the leukocytes. Moreover, when artificial ascites is produced in a monkey stricken with this disease, the injection of this fluid (which contains only leukocytes) produces the disease in other monkeys. When the number of leukocytes is so adjusted that it corresponds to the number of leukocytes in a measured amount of blood, the degree of illness produced in either case will be about the same. These facts indicate that the causative agent is more closely associated with the leukocytes than with the erythrocytes.

If this be so, which type of leukocyte plays the most important part? It is difficult to decide this clinically or morphologically, but as there is little quantitative change in the number of large mononuclears and transitionals, the decrease

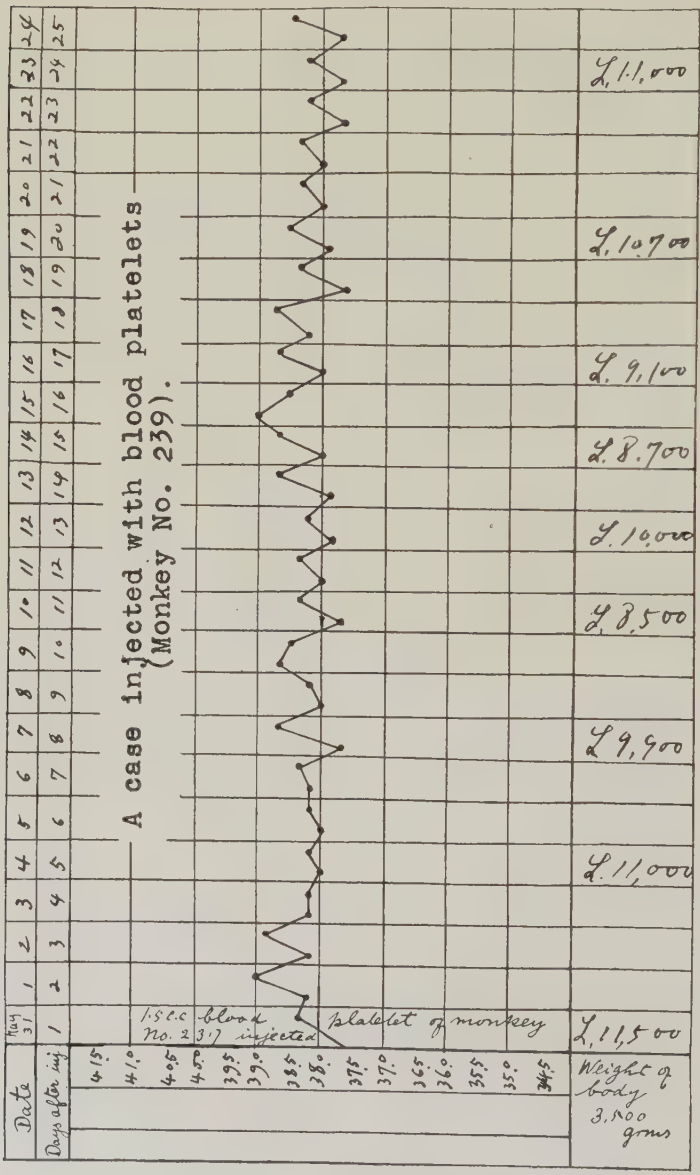

A case injected with blood platelets
(Monkey No. 239).
Date
May 31
Days after inj
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
1.5 c.c blood injected
No. 237
platelet of monkey
Weight of body 3,500 grms
¥ 11,500
¥ 11,000
¥ 9,900
¥ 8,500
¥ 10,000
¥ 8,700
¥ 9,100
¥ 10,700
¥ 11,000

Figure 35

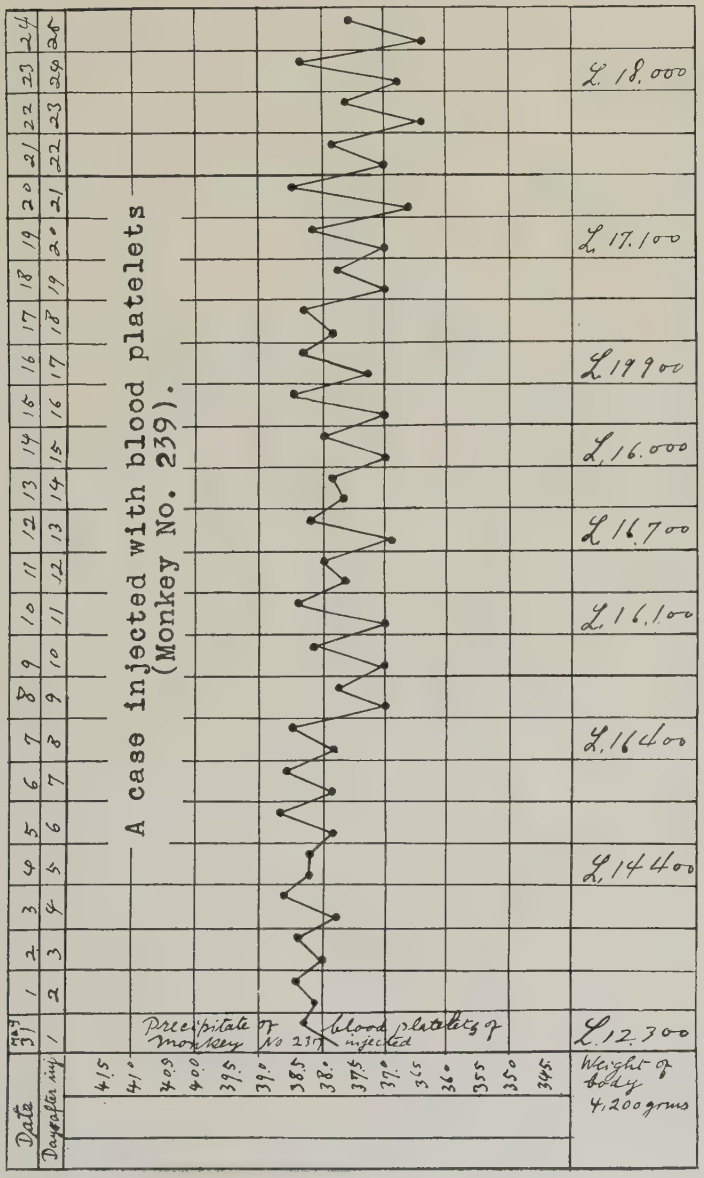

A case injected with blood platelets
(Monkey No. 239).
Precipitate of Monkey No 239
blood platelets injected
Date
Days after inj.
Temp
Weight of body 4,200 grms
£ 12.300
£ 14400
£ 16.100
£ 16.700
£ 16.000
£ 19.900
£ 17.100
£ 18.000

Figure 36

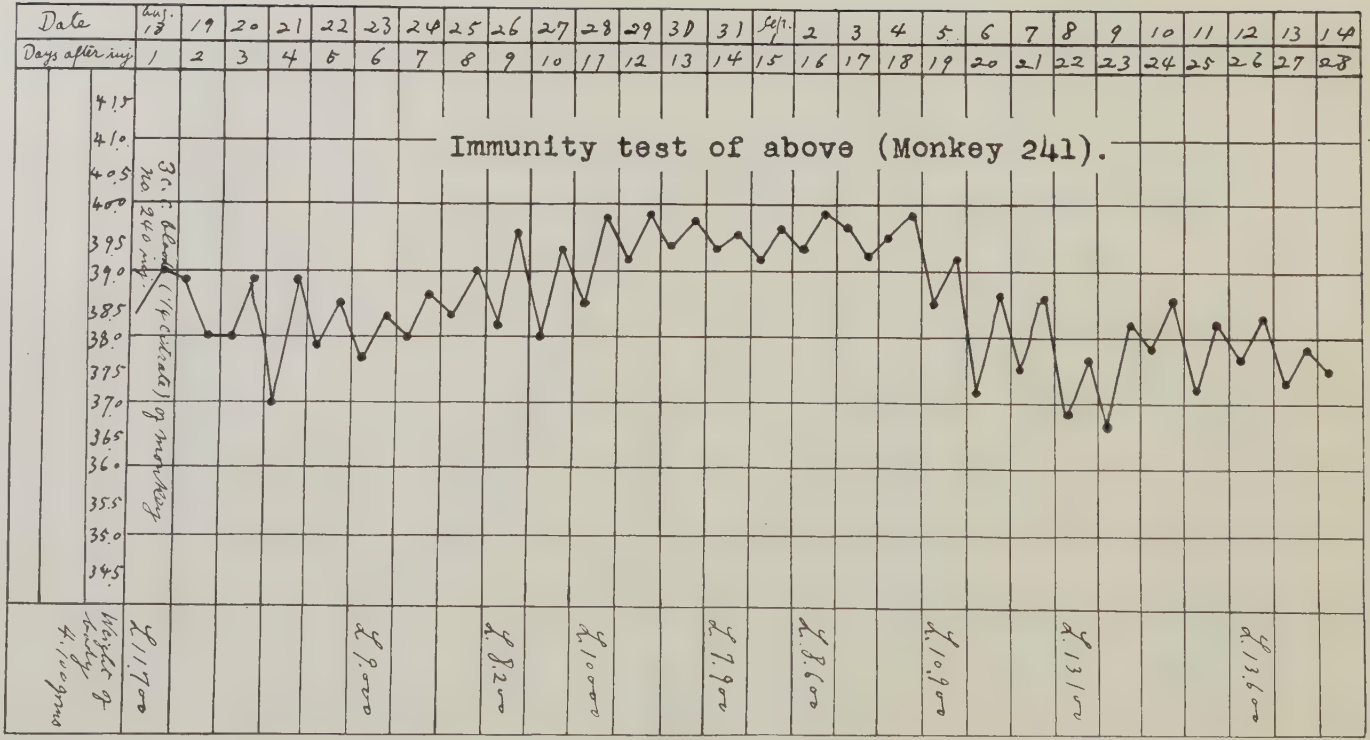

Immunity test of above (Monkey 241).
Date
Days after inj.
Aug. 18
19
20
21
22
23
24
25
26
27
28
29
30
31
Sept. 1
2
3
4
5
6
7
8
9
10
11
12
13
14
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
Weight of monkey 4,100 grms.
4,170 cc
4,200 cc
4,200 cc
4,000 cc
4,190 cc
4,260 cc
4,090 cc
4,310 cc
4,360 cc

Figure 37

in number is seen chiefly in the polymorphonuclears and lymphocytes. Morphological conditions and staining reactions are not much altered. The artificial ascitic fluid contains many polymorphonuclears, with but a trifling admixture of other types. From these facts we may safely conclude that the polymorphonuclear leukocyte has the most important part to play.

Now, if the causative agent has a very close relationship to the leukocyte, what is the nature of this? Does this agent attach itself to the cell surface, or does it maintain mere symbiotic relations to it? We have shown that it does not adhere strongly enough to the cell to prevent its appearing in the supernatant fluid of centrifugated blood or ascitic fluid in fairly concentrated form. A brief comparison of the behavior of the causative agents of typhus and tsutsugamushi fevers may be given at this point. It has been reported by Nicolle and Conseil (129) that the causative agent of typhus is found in the leukocytes, but they encountered several difficulties in confirming this. In 1920, Kusama (90-91) found the agent in blood-platelets which had been separated from the leukocytes and Ségal (168) repeated his experiments in 1922 and confirmed them. Moreover, he found by experimenting with artificial ascitic fluid that the causative agent of typhus has no relation to the leukocytes. The etiological agents of typhus and of tsutsugamushi disease have not as yet been identified beyond any doubt and, as neither of them are filterable, they must resemble each other in their morphological characteristics. It is interesting that the causative agent of tsutsugamushi disease is not found in the blood platelets, while that of typhus fever is.

E. Study of Fluid from Vesicles.

At the time when the eruption on the skin was most marked blistering plasters were applied to five patients and the contents of the vesicles so produced was collected, any cells were removed by centrifugation and the upper, clear fluid was injected into five monkeys. Two of these gave no reaction whatsoever and the other three reacted so slightly that the experiment may be considered quite negative. Therefore, the causative agent is not contained in the fluid of vesicles.

F. Study of the Cerebrospinal Fluid.

Ten c. c. of spinal fluid taken from a patient in the fastigium was injected into monkey No. 65, with entirely negative results.

G. Study of the Urine.

Urine was removed from a patient, under aseptic precautions, eight, ten and fifteen days after the onset and injected whole, or centrifugated and the sediment alone injected into three monkeys (Nos. 6, 31, and 81) without any results.

H. Transmission of the Virus to the Human Fetus.

A woman, nine months pregnant, was delivered of a dead fetus on the twelfth day of her illness. The fetal blood was drawn from the umbilical vein and injected into monkey No. 54, but the animal developed no symptoms. Three c. c. of the blood of this animal was injected into another macaque, which likewise remained unaffected.

I. Resistance of the Virus to Various Physico-Chemical Agents.

a. Effect of Temperature: Three c. c. of citrated blood from an infected monkey was heated to 45° for thirty minutes and injected into monkey No. 93, the animal developing fever after nine days of incubation, but when the blood was heated to 55° for ten minutes and injected into monkey No. 92 there was no "take" and the immunity-test remained negative. Four c. c. of blood from an infected monkey was heated to 50° for ten minutes and injected into monkey No. 83, the result being doubtful, but the immunity test positive.

Thus we find that when blood is heated to 55° for ten minutes the causative agent therein contained is completely destroyed and heating it to 50° would be the maximum temperature it could withstand. This agrees with the findings of Kitashima and Miyajima, who reported that fifteen minutes' heating at 45° is the limit and 50° fatal to the causative agent. They suspended a piece of spleen in salt solution and kept it in an ice-chest for from five to seven days, which resulted in a reduction in the toxicity of the virus, so that a monkey injected therewith did not develop any symptoms.

We incubated 3 c. c. of infectious blood for a week at 37° and injected it into monkey No. 96 without its having any effect. Injection after incubation for five days also proved negative, but three-days' incubation left the virus viable, although the incubation period of the attack produced by its injection into a monkey was prolonged to thirteen days. Incubation at 32° for a week also gave negative results, while at 25° for a week the virus remained viable, but the incubation period of the typical attack produced at its injection was prolonged.

To a mixture of 6 c. c. of normal saline solution and 2 c. c. of rabbits' serum was added 1 c. c. of defibrinated, infectious monkey's blood, the whole being incubated at 20° for two weeks. When 8 c. c. of this mixture was injected into monkey No. 190, it came down with a slight fever after an incubation period of twelve days. The fever lasted five days, with a leukopenia of 7,100, the original count having been 11,500. The immunity test was positive. Thus the virus is viable for two weeks at a low temperature. When 2 c. c. of the infectious blood was injected into a monkey after having been kept for ten days at 0° - 2°, the result of inoculation was negative, but blood injected after one week's storage at this very low temperature gave a positive "take."

The virus is, therefore, viable for three to five days at 37°, seven to ten at 0° - 2°, and over a week at 25°.

b. Effect of Drying: Three to five c. c. of infectious monkey's blood was poured into a porcelain dish, avoiding direct sunlight and was allowed to dry as rapidly as possible. Portions of this were then placed in a drying apparatus for 12, and 72 hours and 7, and 16 days respectively. After each period of drying it was diluted with saline solution and injected into monkeys, all of which gave negative results. The first injection had been dried for twelve hours only, yet it was no longer viable.

c. Effect of the Addition of Chemicals: Kitashima and Miyajima reported that the virus lost its toxicity in pure glycerol, and that the addition of water, to lake the corpuscles, destroyed it also. We added 2 c. c. of 1% mercuric chlorid, 1% phenol, and 2% potassium hydroxid solutions to an equal quantity of infectious blood and injected the mixtures into

three monkeys, all of which remained unaffected. Ascitic fluid was mixed with an equal amount of glycerol and the mixture injected into a monkey which was also not affected. When bile was added to this blood, in equal quantity, and incubated for one day, injection of the mixture was innocuous. Bile and ascitic fluid mixed were also harmless.

J. Is the Virus Still Viable in the Blood of Patients After Death?

In 1917 one of the patients died after a very severe attack of the disease on the ninth day after its onset; six hours post mortem the spleen was removed and 0.8 gm. emulsified and injected into monkey No. 82. The animal developed no fever and the immunity test was negative; but after injecting stock virus into the animal it developed the disease after eleven days' incubation. As the spleen of experimental animals contains the virus during the fastigium of the disease, it is certain that the human spleen must also contain it; this being so, the causative agent must have lost its virulence, in this case, very shortly after the death of the patient.

K. The Effect of Distilled Water on the Virus.

To 6 c. c. of defibrinated, infectious blood we added 2.5 c. c. of distilled water, the mixture was incubated at 25° for twelve hours and was then injected into monkeys Nos. 180 and 181. All the animals were unaffected. This agrees with the findings of Kitashima and Miyajima, who explain that the hemolytic action of water lakes the erythrocytes and conclude that this indicates a very close relationship between them and the virus; but may it not prove, instead, that the products of hemolysis exert an harmful influence upon the virus, the mere mechanical destruction of the corpuscles having little significance in itself? To artificially-produced ascitic fluid an equal amount of distilled water was added, the mixture incubated at 25° for one day and then injected into two monkeys, Nos. 301 and 302. Both developed a typical attack after an eight-day incubation period. To the precipitate of this ascitic fluid we added a like amount of distilled water and injected the mixture into monkey No. 312, the animal developing a typical attack with a somewhat prolong-

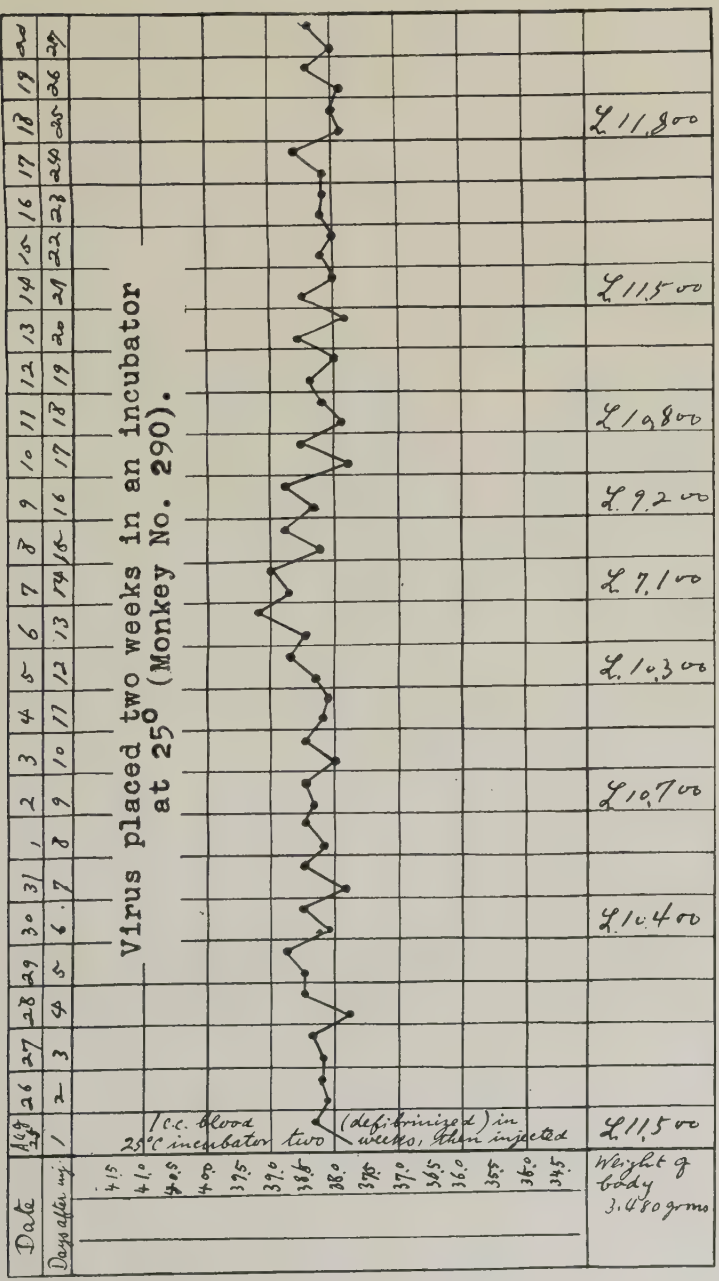

Virus placed two weeks in an incubator at 25° (Monkey No. 290).
1 c.c. blood
25° C incubator two
(defibrinized) in
weeks, then injected
Date
Days after inj.
Aug 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
4.15 4.1 4.05 4.00 3.75 3.70 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45
£11,500
£11,300
£11,500
£10,800
£9,200
£7,100
£10,300
£10,700
£10,400
Weight of body 3,480 grms

Figure 38

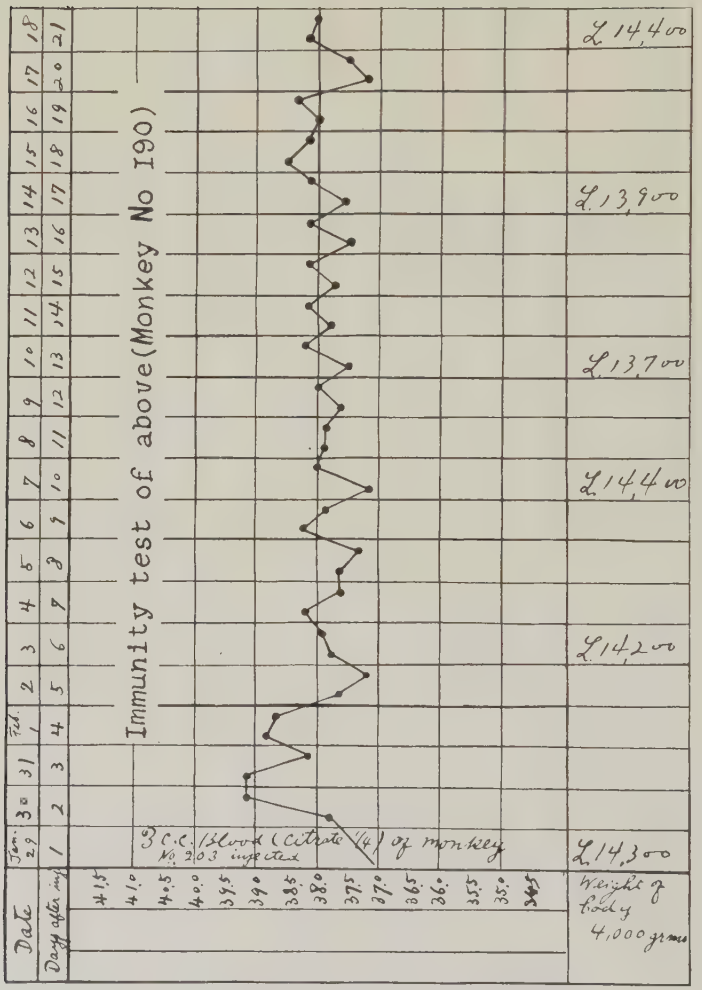

Immunity test of above (Monkey No 190)
3 C.C. Blood (Citrate (4)) of monkey No 203 injected
Date
Day after inj.
Jan. 29
Feb. 1
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
¥14,400
¥13,900
¥13,700
¥14,400
¥14,200
¥14,300
Weight of body 4,000 grms

Figure 39

ed incubation period. Thus, the destruction of erythrocytes has no relation to the viability of the virus other than setting free hemolytic products which act harmfully upon it. The lack of any influence upon the virus on the part of distilled water is very interesting.

L. The Effect of Neosalvarsan and Quinine on the Virus.

Since arsenic preparations have a specific action upon trypanosomes and spirochetes, it is important to ascertain whether or not a virus of unknown composition possesses arsenic receptors, not only for reasons of therapeusis, but also for a better understanding of the nature of that virus. Ogata, Kitashima, and Miyajima reported that atoxyl had no action whatsoever upon tsutsugamushi disease, and Miyajima has reported that salvarsan is also of no use in combating it. As we had no detailed reports of our own on this subject, we desired to repeat these experiments and selected neosalvarsan for the sake of convenience, injecting it into the veins at the elbow in rather concentrated form. Two patients both of them strong farmers received injections in the fastigium of the disease. After injection their temperature was taken every three hours and their blood examined every six hours afterwards.

Case I. Male, 28. Ten days after onset he had a fever of 39° - 40.5°. He received 0.4 gm. of neosalvarsan at 6 P. M. on August 14th, 1914.

Case II. Male, 34. Nine days after onset he had a fever of 39° - 40.6°. He received 0.4 gm. of neosalvarsan at 6 P. M. on September 17th, 1914.

Temperatures of Case I. following neosalvarsan injection:

14th day of disease	T°	Time 15th day of illness	T°
Before injection	39.4	3 P.M.	39.6
9 P.M.	39.8	6 P.M.	39.2
12 P.M.	39.9	9 P.M.	39.2
3 A.M. (15th day)	39.8	6 A.M. (16th)	39.7
6 A.M.	39.5	12 M.	39.6
9 A.M.	39.7	9 P.M.	39.9
12 M.	39.9		
Leukocyte counts:			
6 P.M. (14th day)	3,306	12 P.M.	4,460
6 A.M. (15th day)	4,372	12 M.	3,562

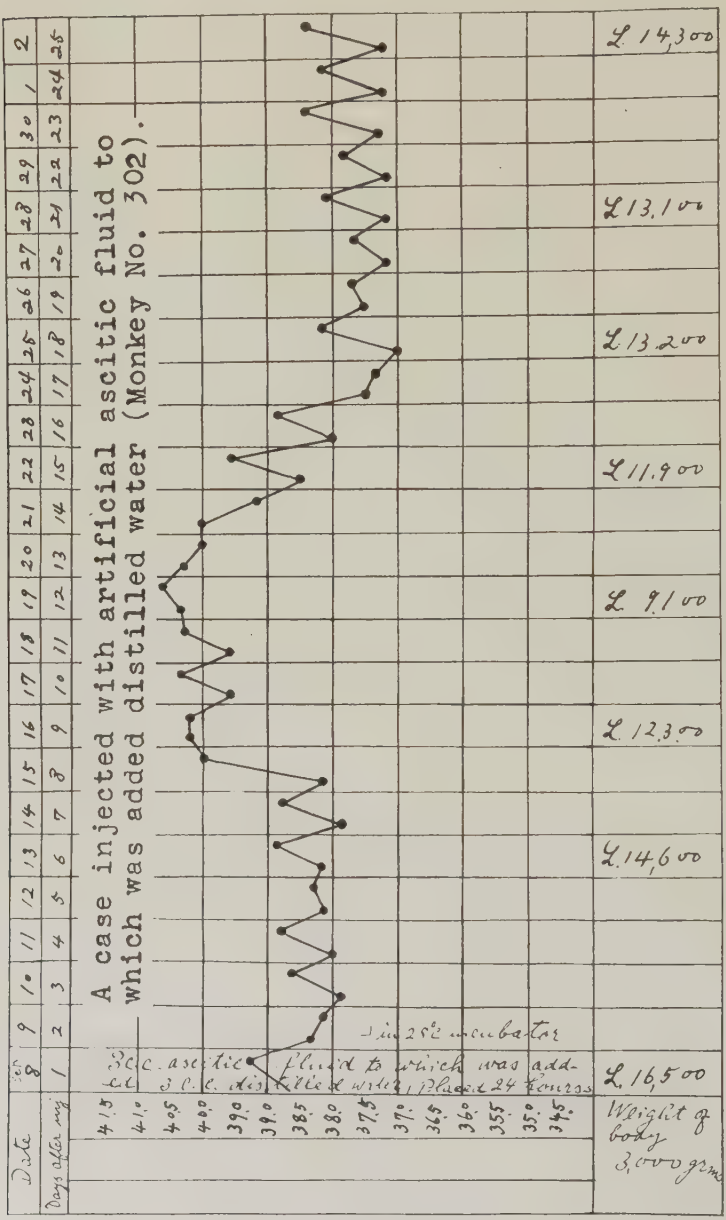

A case injected with artificial ascitic fluid to which was added distilled water (Monkey No. 302).
Date
Days after inj.
Dec 8
1 2 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 1 2
41.5
41.0
40.5
40.0
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
3cc. ascitic fluid to which was added 3 c.c. distilled water, placed in 28° incubator 24 hours
Weight of body 3,000 grms.
¥ 16,500
¥ 14,600
¥ 12,300
¥ 9,100
¥ 11,900
¥ 13,200
¥ 13,100
¥ 14,300

Figure 40

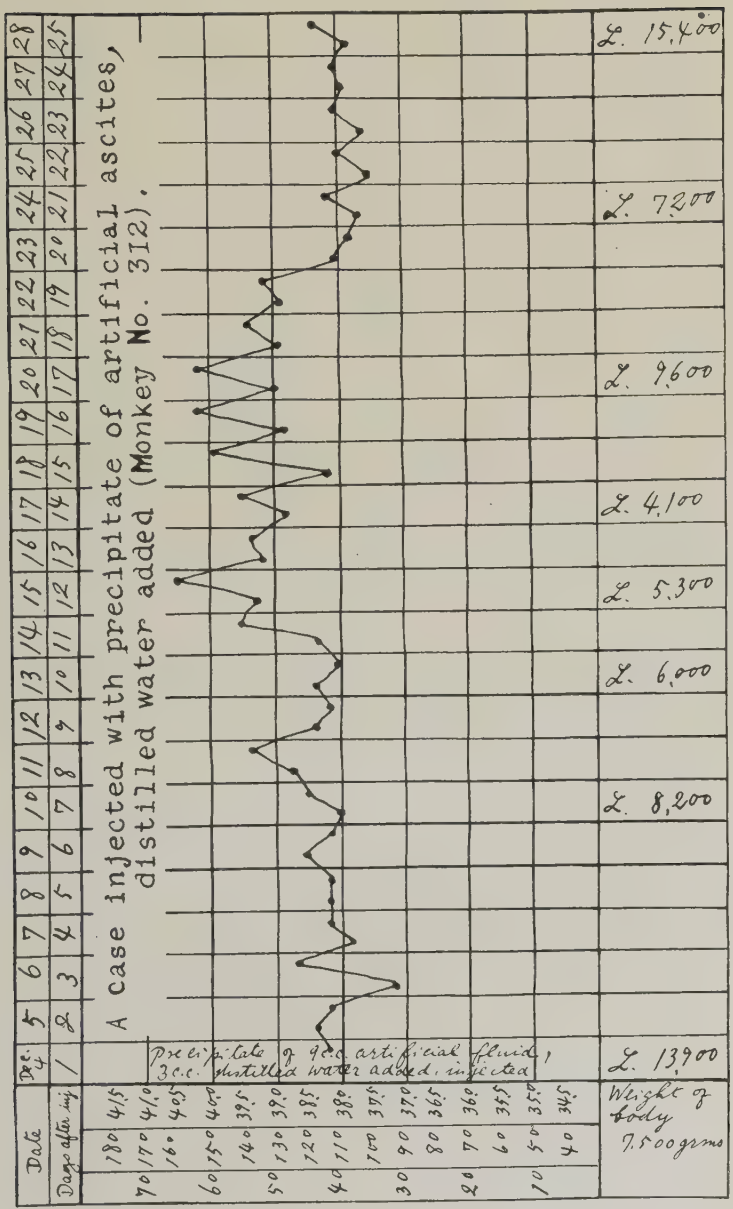

A case injected with precipitate of artificial ascites, distilled water added (Monkey No. 312).
Precipitate of 9cc. artificial fluid, 30 c.c. distilled water added, injected
L. 15,400
L. 7200
L. 9600
L. 4,100
L. 5,300
L. 6,000
L. 8,200
L. 13,900
Weight of body 7,500 grms

Figure 41

The temperatures of Case II. were as follows:

	T°	Time	T°
17th day before injection	39.3	6 A.M. (19th day)	39.7
9 P.M.			
6 A.M. (18th day)	39.8	9 A.M.	39.4
9 A.M.	39.2	12 M.	39.6
12 M.	39.5		
3 P.M.	40.1	3 P.M.	39.8
6 P.M.	40.4	6 P.M.	39.7
9 P.M.	40.0	9 P.M.	39.2
		12 P.M.	39.8
Leukocyte counts:			
6 P.M. (17th day)	6,060	12 P.M.	5,960
6 A.M. (18th day)	7,900	12 M.	8,420
6 P.M.	6,010		

The injection of neosalvarsan had no influence on the temperature of these patients, but the number of leukocytes increased in both cases and reached its maximum six to eighteen hours after injection. Then it gradually decreased and, 30 - 40 hours after injection reached its original figure. There was no particular change as to the variety of the leukocytes. The curves and leukocyte-counts are shown in Figs. 42 and 43.

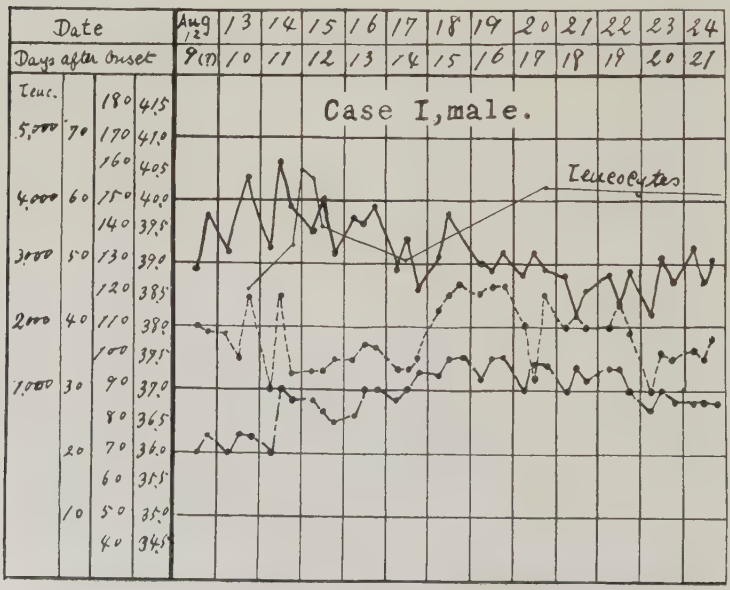


Figure 42

In order to determine the relation of neosalvarsan to the increase in leukocytes, control cases in which the Wassermann reaction was strongly positive were treated with concentrated neosalvarsan, injected into the veins at the elbow. The temperature and the number of leukocytes 24 hours after the injection were as follows:

Case I. M., 31. Amt. injected, 0.32 gms.

Time	T°	Leukocyte count
6 P.M. before injection	37.1	6,500
6 A.M.	37.0	6,760
12 M.	37.4	6,420
6 P.M.	36.9	6,000

Case II. F., 31. Amt. injected, 0.28 gms.

Time	T°	Leukocyte count
6 P.M. before injection	37.5	8,920
6 A.M.	36.8	8,420
12 M.	36.1	7,560
6 P.M.	37.1	8,210

Case III. M., 51. Amt. injected, 0.4 gms.

Time	T°	Leukocyte count
6 P.M. before injection	36 8	6,680
12 M.	36.8	8,020
6 P.M.	36.9	8,860

The foregoing control cases show that in two the injection

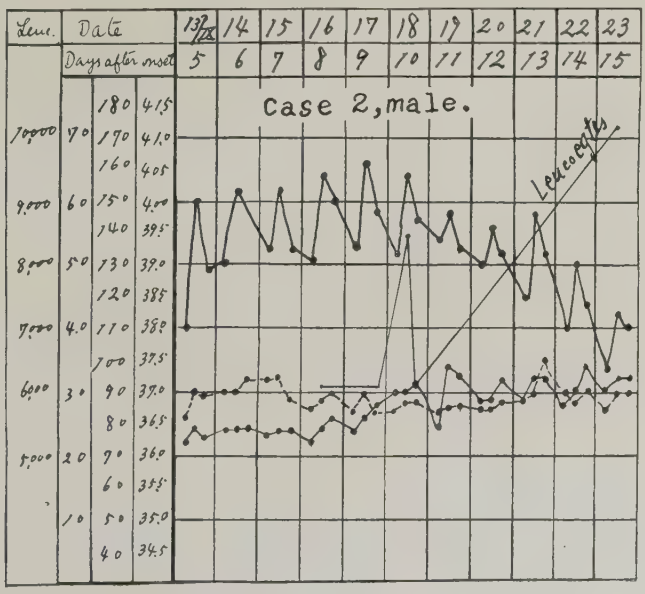


Figure 43

had no influence upon the number of leukocytes, but in the third there was a marked increase. The temperature, on the other hand, was not influenced.

Quinine hydrochloride in amounts of 0.7 to 1.0 gm. had no effect upon the temperature.

These two preparations have no effect upon tsutsugamushi disease and our results are thus completely in accord with those of Ogata, Kitashima and Miyajima. It is worthy of note that in two cases we had a transient leukocytosis, the reason for this being that the arsenic had stimulated the blood-forming organs as indicated by one of the three control cases. As we have already noted changes in the bone marrow at necropsy, such as the appearance of phagocytes and small necrotic foci, we may conclude that it is in a state of more or less increased sensibility and easily stimulated by arsenic.

M. The Effect of Immune Serum upon the Virus.

It has been repeatedly stated that the immunity bestowed by this disease is relatively feeble and it is therefore important to determine the effectiveness of the action of immune bodies upon the virus, in order to gauge its efficiency in combating the disease. We tested it out with men and animals in a series of experiments a description of which follows.

In 1915, we took blood from three convalescent patients and injected the sera into others.

Case I. A man of thirty was intravenously injected with 3 c. c. of this serum on the eleventh, twelfth, and thirteenth days after the onset of the disease, but he died on the nineteenth.

Case II. A woman of twenty-seven received 2 c. c. of the serum on the eighth day and 2.7 c. c. on the ninth after onset, without the course of the illness being at all influenced.

We used rather small doses of immune serum in these cases and its effectiveness might thus have been considerably lessened, therefore the following experiment was done to see what effect, if any, larger doses might exert on the virus. On July 19th, 1917, a monkey (No. 174) which had been inoculated with a patient's blood and had had a typical onset ten days later, was bled five days after the subsidence of fever. Its serum was mixed in the ratio of five to ten parts of this to one of the blood of monkey No. 93, which was in its fastigium. The mixture was left at room temperature for an hour and was then injected into two monkeys, Nos. 101 and 102. Both had a typical attack after a nine-days' incubation period. Kitashima and Miyajima obtained similar results by emulsifying the spleen and mixing ten grams thereof with 30 c.c. of immune serum, injecting this into monkeys. These developed typical attacks of the fever.

These experiments indicate the feebleness of the protection exerted by immune serum, but as we said before, the virus of the disease is in close relationship to the leukocytes and very little of the virus floats in the plasma, where it might be attacked by the immune bodies, hence the action of immune serum is indirect rather than direct.

N. Experiment on Natural Infection.

Kitashima and Miyajima, Nagayo, and Hayashi have all reported that monkeys brought into an endemic region may spontaneously contract the infection and we, too, have made a few experiments along this line.

On August 14th, 1917, we kept monkey No. 78 in an endemic region for several hours, finding ten akamushi attached to the eyelids of both eyes at the end of that time. The macaque had an attack of fever after one week's incubation and the fever lasted ten days with marked leukopenia. There were still two akamushi attached to the eyelids five days after exposure. Nine days after exposure one bite lesion was slightly swollen and covered with a crust, but the others were merely slightly reddened. The superficial nodes were slightly swollen. The immunity test was positive.

Several guinea pigs were also brought into the endemic region and after some time we found that there were a few, to several hundred akamushi attached to their ears. Two of the animals, that seemed to have some fever, were killed two weeks after they had been exposed and 3 c. c. of their blood mixed with 0.8 gm. of their emulsified spleens were injected into monkey No. 91, which developed a typical attack with a ten-day period of incubation.

White rats were also tested and we found a few to several hundred akamushi attached to the conchae of their ears. Five of them were killed two weeks later and 1.5 gm. of emulsion of their spleens was injected into monkey No. 67, which developed a typical attack ten days later. (Fig. 44).

Three rabbits were also exposed in the endemic region, one of them had several akamushi attached to the canthus of the right eye and a few on the left ear. (Fig. 45). It was killed two weeks later, 4 c. c. of its blood producing a typical attack in monkey No. 68, with an incubation period of twelve days.

Natural infection of monkeys, guinea pigs, rabbits and white rats through the bite of akamushi is therefore possible. The guinea pigs and rabbits had more or less atypical fever and had no other symptoms of the disease; but injection of their blood, or splenic emulsion into monkeys produced typical attacks of fever in these animals

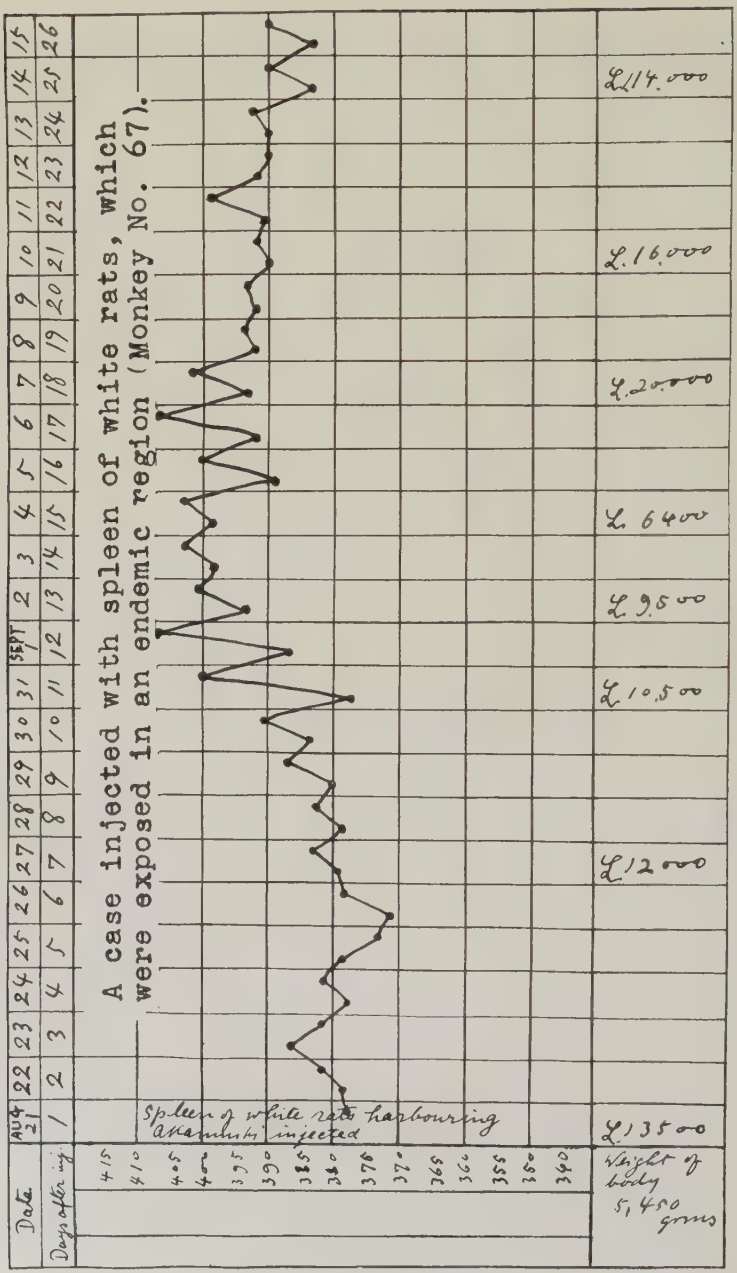

A case injected with spleen of white rats, which were exposed in an endemic region (Monkey No. 67).
Spleen of white rats harbouring Akamushi injected
Weight of body 5,450 grams
£13500
£12000
£10500
£9500
£6400
£20000
£16000
£14000

Figure 44

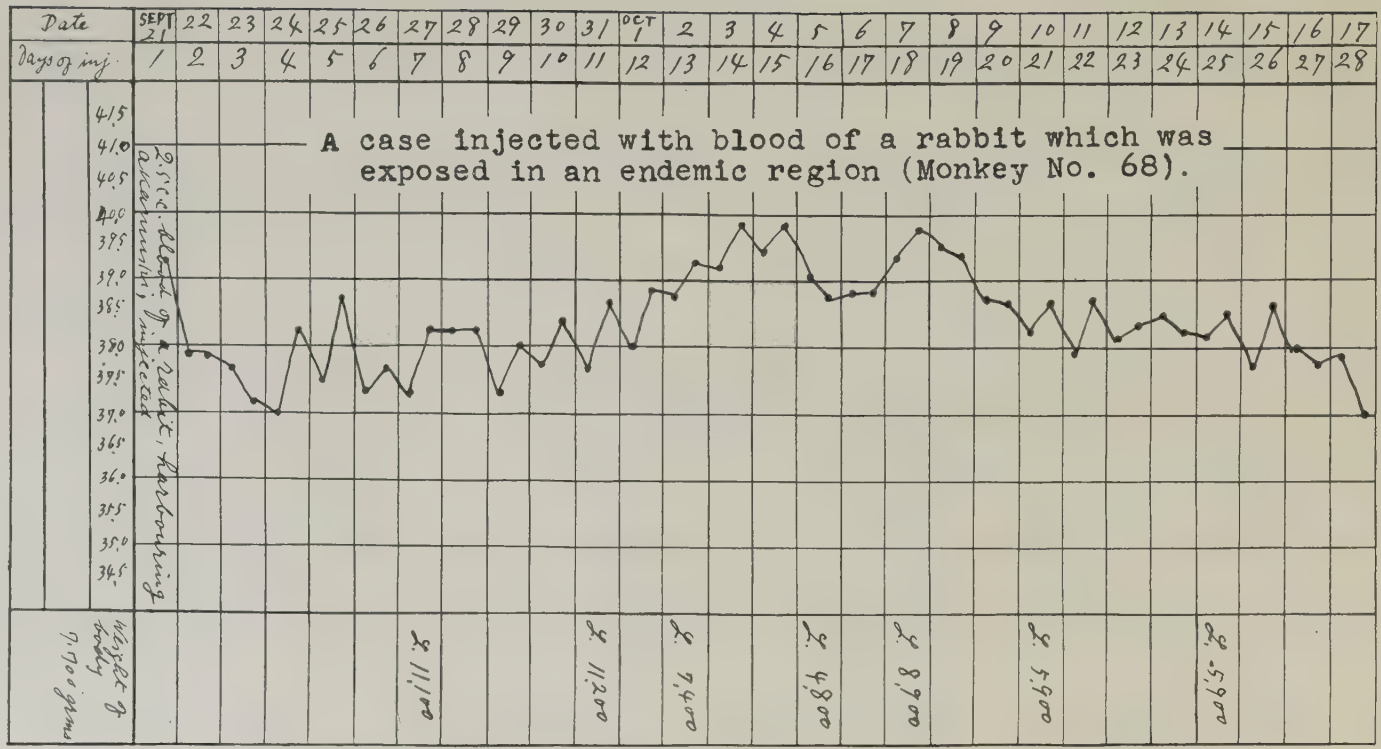

A case injected with blood of a rabbit which was exposed in an endemic region (Monkey No. 68).
Date
Days of inj.
SEPT 21 22 23 24 25 26 27 28 29 30 31 OCT 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
41.5 41.0 40.5 40.0 39.5 39.0 38.5 38.0 37.5 37.0 36.5 36.0 35.5 35.0 34.5
2 c.c. defib. blood of Rabbit, Hartoum/41, intracecal
Weight of body 1,700 grams
x 1,100
x 1,200
x 1,400
x 4,800
x 8,600
x 5,600
x 5,500

Figure 45

4. The Relation of Tsutsugamushi Disease to Akamushi and Field Voles.

Two cases are reported by Kitashima and Miyajima and one by Hayashi, in which the infective akamushi have bitten human beings and actually caused infection with tsutsugamushi disease and it is generally believed that the akamushi has a close relationship to this malady, although actual clinical observations of this fact has been very rare. In 1914 we had experience of this sort with three cases, one in 1915 and a fifth in 1916. The following are the case-histories.

Case I. M., 30 (1914). This man had not been in the endemic region, coming into it on August 20th, for the first time. On September 1st, he felt itching on the inner side of his left thigh near the scrotum and, on examination, found an akamushi attached to the skin and removed it. Later the bite became swollen and necrotic and there was regional swelling of the lymphnodes with tenderness. On the 9th, he complained of headache and fever and entered the hospital on the 12th.

Case II. M., 57 (1914). This man had frequently been bitten by akamushi, but had never had the disease before. On August 15th, he came to the endemic region and in the evening of that day, on touching his abdomen, felt an itching sensation. On examination he found an akamushi attached to the skin and removed it. Later the bite became swollen and on the 25th he developed the disease, with a mild onset of slight headache.

Case III. M., 41 (1914). This patient had had tsutsugamushi disease at the age of four and again at thirty. On the second occasion he found an akamushi at the side of his left elbow and the course of the attack that followed the bite was very severe. He had already been bitten frequently by akamushi, this season alone he was twice attacked. On September 20th he was bitten in the left axilla and ignored the bite. Later it developed into a primary lesion and, on the 20th, a typical attack of the disease came on, with slight headache, lassitude and fever.

Case IV. F., 23 (1915). This woman had never been bitten before. She had been in the endemic region for about two hours on the morning of June 20th, and noticed itching in two places in the right axilla. One of these was cauterized with phenol, but the other was left untreated, developing later into a primary lesion, and on July 7th, she came down with the disease, complaining of headache, loss of appetite and lassitude.

Case V. F., 41 (1916). (Fig. 46). This was not one of our cases, the specimen being sent in by a physician who had had long experience in the endemic region. On removing the tissue around the bite he noticed an akamushi still attached to the skin. The patient had been bitten in the right axilla, coming several hours later to the physician, who excised the surrounding tissue. In spite of this, a primary lesion developed and after nine days the patient had swelling of the regional nodes and a typical onset of symptoms of the eleventh day. The skin removed and sent to us still had the akamushi in situ and there can be no doubt that it caused the infection. It was 0.263 x 0.1753 mm. in size, its color was bright red, and a comparison of various parts of its body with those of typical akamushi proved it to be of the same species.

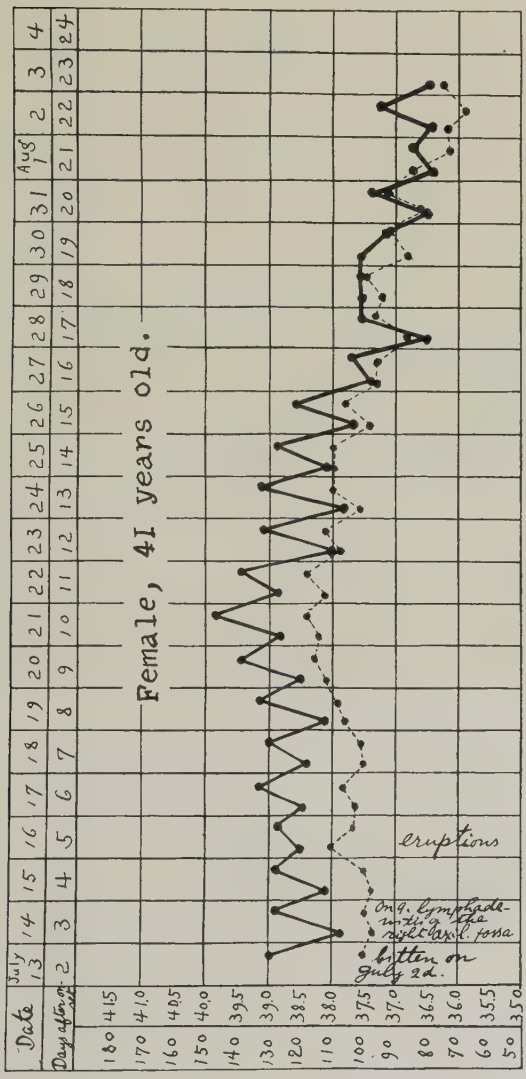

Female, 41 years old.
Date
July 13
Aug
Day after sickness
bitten on July 22.
eruptions
Enl. lymphade-nitis of the right ax. l. fossa

Figure 46

There is, therefore, an undoubtedly intimate relation between the bite of akamushi and the tsutsugamushi disease. The reason why we do not see many akamushi still attached is because they are usually in more or less hidden portions of the body and the patients overlook their presence; had they carefully searched they would have found these mites more frequently.

We shall now describe the relation of akamushi to the Japanese field vole. As akamushi lodge in large numbers in the ear-conchae of these animals in the endemic localities, many investigators have already noted a relationship between them. Kitashima and Miyajima reported that the injection of the spleen of field voles harboring many akamushi into monkeys did not produce tsutsugamushi disease, but if a patient's blood were injected into voles and transferred to others three times, the blood of the last vole to be injected would produce the disease if injected into a monkey. Hence they said that field-voles harbor the virus, but do not have the disease; they are carriers and the akamushi get the virus from them. Hayashi injected the spleen of field voles into four monkeys, during the endemic season, and all these developed fever. A year later he injected the spleen of field voles mixed with that of a patient, but the result was negative. From this he concluded that the spleen of the voles injected during the previous year had contained the causative agent.

In our several experiments with injections of the spleen of the field vole during the endemic season, all those monkeys injected with the emulsion of swollen spleen developed typical attacks, as Hayashi reported. It is important from the epidemiological viewpoint to determine whether or not the field voles carry the virus during the summer only, or during other seasons as well; we therefore performed the following experiments, one with voles caught during the spring and the other with voles caught during the autumn.

A. During March and April, 1921, we collected forty field voles and emulsified twenty of the spleens weighing over 0.1 gm. each and showing a dark-red color; the emulsion was injected into two monkeys, Nos. 208 and 209. (Fig. 47). These had an attack of fever, after ten and eleven day periods of incubation, No. 208 dying during defervescence. Necropsy showed slight swelling of the spleen, general swelling of the lymphnodes and marked gastritis.

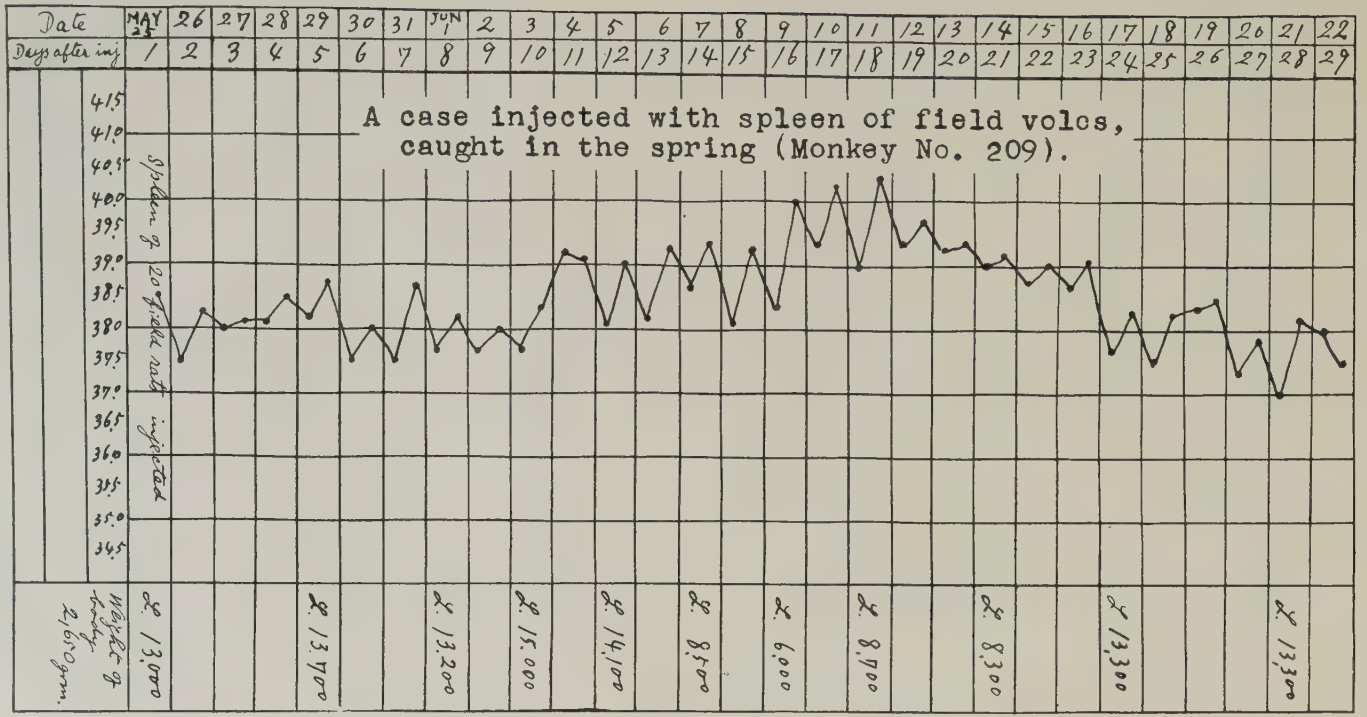

A case injected with spleen of field voles, caught in the spring (Monkey No. 209).
Date
Days after inj
MAY 25
JUN 1
41.5
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
34.5
Spleen of 20 ticks not injected
Weight of body 2,610 gm.
L. 13,000
L. 13,700
L. 13,200
L. 15,000
L. 14,100
L. 8,500
L. 6,000
L. 8,700
L. 8,300
L. 13,300
L. 13,300

Figure 47

B. In October, 1917, after cases had ceased to occur, twenty-two voles were collected from the endemic locality and the spleens of these injected into two monkeys (Nos. 97 and 108). Both animals had a typical onset of fever after an eleven-day latent period. One akamushi was found in the ear concha of each of four voles.

These two experiments prove that field voles living in the endemic region carry the causative agent not only during the summer but also through the winter into the spring, which has an important bearing on the infectivity of the akamushi.

The next question to be answered is whether or not the bite of newly-hatched akamushi larvae would cause infection with the virus of tsutsugamushi disease. In 1915, independently of Nagayo and Miyajima, I found the adult form of akamushi and established its life cycle. Since then we have been able to breed larval mites from adults in captivity. We carried out infection experiments in monkeys with these laboratory-bred larvae. In 1918 Kitashima and Miyajima raised 155 larvae bred from adults in captivity and allowed them to attach themselves to four monkeys. One, with 28 akamushi attached, became infected with tsutsugamushi disease; but five, injected with an emulsion of 115 akamushi larvae and 20 nymphs gave four negative and one doubtful take.

In 1918 I allowed akamushi larvae bred in the laboratory to attach themselves to monkeys. Two monkeys, which had 100 and one that had 42 of them attached, were not infected. The next year the test was repeated and gave positive results. The adult akamushi used in this experiment were caught during the spring and autumn of 1917 and 1918 in the endemic region. In this experiment the number of akamushi attached to the monkeys were 133, 222, and 308 respectively. The monkeys having the first two numbers attached were unaffected, while the third became infected. The following is a detailed description of that monkey.

Monkey No. 122, (Fig. 48). On July 6th, akamushi which had been bred in an incubator, were allowed to attach themselves to this monkey; the next day about fifty were found around the face and eyes and several in the left ear. On July 12th, a yellow crust was found on the forehead and vesicles on the left eyelid. The former remained unchanged in appearance for about ten days, without any swelling appearing in the regional lymphnodes. On the fourteenth day fever developed and ran a typical course, with marked leukopenia at the beginning of the attack. On the seventeenth day blood was drawn from

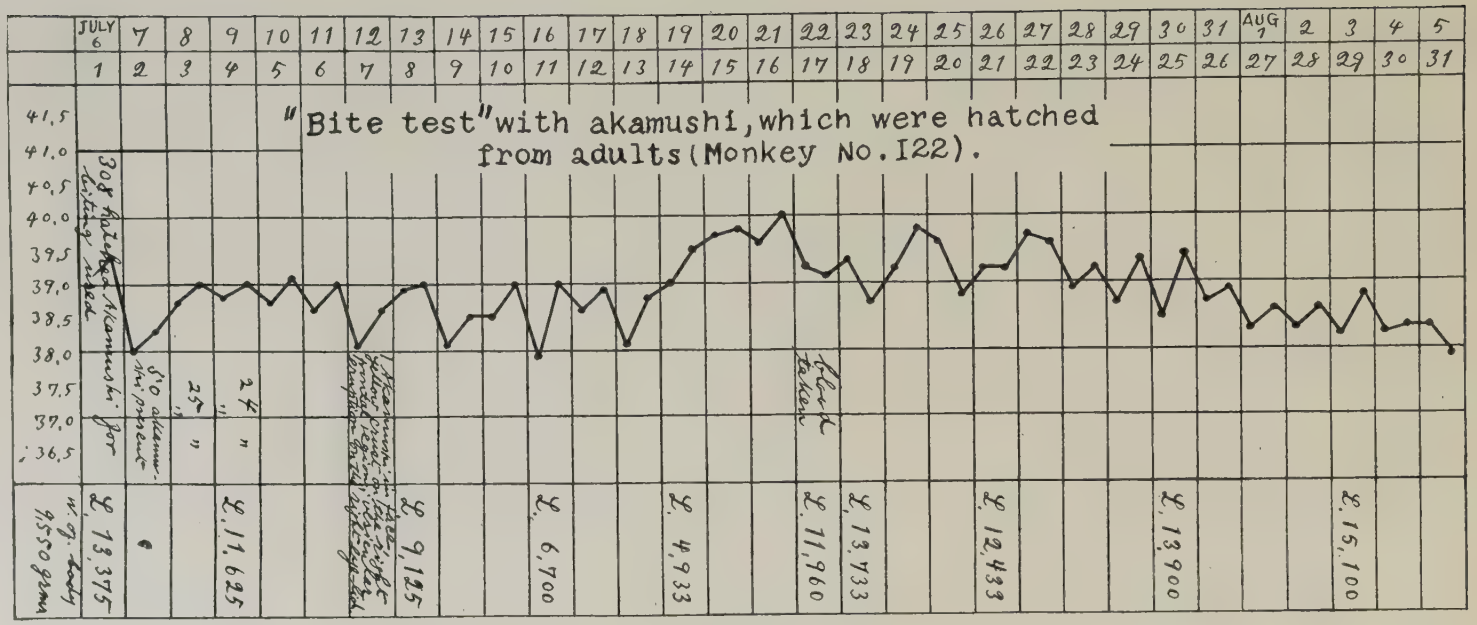

"Bite test" with akamushi, which were hatched from adults (Monkey No. I22).
JULY 6
AUG 7
41.5
41.0
40.5
40.0
39.5
37.0
38.5
38.0
37.5
37.0
36.5
30 akamushi biting this area
10 akamushi present
25 " "
24 " "
Blank taken
£ 9,125
£ 6,700
£ 4,933
£ 11,960
£ 13,733
£ 12,433
£ 13,900
£ 15,100
£ 11,625
£ 13,375
9550 gms

Figure 48

the femoral vein, mixed with citrate solution and injected into another macaque, No. 124, which developed a typical fever after twelve days of latent period, with leukopenia and a positive immunity test.

This experiment demonstrates that the yellow crust seen on the forehead was formed over the bite from which the monkey contracted the disease. It is known that the bite of an infective akamushi produces a primary lesion, as shown by the experiments on natural infection and since we found only one primary lesion in the entire experiment, it may be concluded that there was but one infective akamushi among the total of 663 larvae. When this fact is taken into consideration, in connection with the experiments of the previous year in which only 242 akamushi were used, it may be stated that very few of the laboratory-bred akamushi harbor the virus. This is also in all probability true of the akamushi naturally bred in the endemic region, very few of which actually serve as vectors for the disease; but the laboratory mite carries a smaller quantity of the virus than do these which prolongs the incubation period in the monkey beyond the limits usually noted after bites inflicted under normal, outdoor conditions. The actual reason for this requires further investigation, although it may bear some relation to the conditions of soil, temperature and nourishment of the animals which harbor the virus, mainly the field voles.

This series of experiments clearly establishes that the virus-bearing akamushi is the vector of tsutsugamushi disease. The next point to investigate is whether it is necessary to have an akamushi bite a victim or whether emulsified akamushi will produce the disease after being injected into its skin. The naturally-grown akamushi were washed first with salt solution and then emulsified; the substance of sixty being inoculated into one monkey, of 100 into another, and the following day 60 into both animals; but there was no take. We have seen, in the previous experiment, that only a very few akamushi harbor the virus and there may not have been any carriers among the 220 akamushi used. Therefore, in the following experiment, we employed a larger number.

When the conchae of the ears of field voles were cut off and left alone, the akamushi detached themselves and were then collected, washed with salt solution, or hydrogen peroxide, and emulsified. This experiment was repeated three times, one monkey was inoculated with

the emulsion of 200 mites, one with that of 1,000, and a third with that of 1,200; in the two last we obtained takes. With 1,000 akamushi the incubation period was nineteen days, with 1,200 it was fifteen. The transmission of the blood of these, to two other monkeys produced positive takes in eight and nine days respectively.

Thus the disease may be transmitted by injecting the akamushi in toto, without the necessity of its biting the victim. Such akamushi either contain the virus in their bodies or acquire it from field voles by sucking their blood. Artificial transmission prolonged the period of incubation, which is interesting, but once a monkey contracts the disease this may be transferred to another monkey without the latent period being lengthened.

The result of experiments on other types of akamushi were as follows:

Type B. An experiment with 150 larvae attached to an animal turned out negative. When 1,400, taken from the conchae of field voles, were emulsified and injected into monkey No. 165, the animal had a typical attack after a fifteen days' latency. Transmission from this animal to another produced an attack nine days later. Thus the character of the virus carried by this type is the same as that found in the true akamushi.

Type C. On two occasions 1,620 of this type, and once 1,100, were emulsified and injected into monkeys with a doubtful result. Again injecting 2,200 emulsified mites the results remained negative.

Type D. We emulsified 2,100 and 1,100 of this type and injected them into a monkey, also allowing 90 newly hatched larvae to attach themselves to its skin, but there was no result.

Type E. Here again, the result of the injections of 1,500 emulsified larvae of the E' Japanese type was quite negative.

From these experiments we find that besides the true akamushi, Type B is the only one to harbor the virus of tsutsugamushi disease.

To determine whether the adult akamushi carry the virus we collected them from the endemic region, washed them either with peroxide of hydrogen or 70% alcohol for a short time, and then rinsed them several times in normal saline solution to remove surface bacteria. They were then emulsified and injected into monkeys. In 1918 we collected the adult akamushi from an endemic area near the Shinano river at various seasons of the year. The first experiment was done with 270 adult akamushi and the second with 170, while a third and fourth were performed with 1,200 each. All the

injected animals remained unaffected and their immunity tests were uniformly negative.

Nagayo, however, reported at the seventh meeting of the Japanese Pathological Society, that he had collected adult akamushi in May and between November and December, in the endemic region in Yamagata prefecture and obtained two takes after injecting from 240 to 400 adult mites on four occasions. He transmitted the disease from one of the positive monkeys to a lot of five monkeys and obtained positive takes in three, the other two remaining negative. Two of the positive monkeys showed incubation periods of ten days and the third one of fourteen. The symptoms in these infected monkeys were very slight. On transmission of the disease from these to other monkeys, he obtained distinct symptoms. His results are directly at variance with ours and the reason for this is obscure, as we had a sufficient number of akamushi for our experiments, collected at different seasons of the year, and from the most heavily infested locality. Since this experiment is of great importance in the study of the virus of this disease, we repeated it the same year.

This time we collected the adults along the banks of the Akano River, which is another endemic region, during the months of May and June, and we performed six experiments with this material: four with 300 mites each, one with 400, and one with 550. One of the monkeys, injected with 300 adult akamushi, gave a negative result, but a positive immunity test and showed leukocytosis. Therefore this was a "doubtful take." The second died of strangulation twenty-six days after inoculation, so that no immunity test was made. The third also died of strangulation, ten days after injection, but showed no fever up to that time. The fourth one was negative and had a negative immunity test, although there was distinct leukopenia after a ten day latent period. The monkey that received 400 mites had uniformly negative results, with a negative immunity test after eight days. Inoculation with infective blood, however, produced typical symptoms after 8 days' incubation. The animal receiving 550 mites also gave no result and a negative immunity test developing the disease after nine days incubation on subsequent incubation.

In the foregoing six experiments, save for one macaque that died too early for results to be noted, all the rest were unaffected. One, however, gave a positive immunity test. I also tried this out with 200 adult akamushi collected in Yamagata prefecture, but with a negative outcome.

Thus we performed ten experiments with adult akamushi collected from different localities and extending over two years, without success. We also experimented with nymphs of akamushi, the first time with an injection of 55, the second with 500, again without success. Thus, we collected 4,990 adults and 550 nymphs and repeatedly carried out experiments with these, which were all negative. Despite this we may safely conclude that the adult akamushi in Niigata prefecture does not cause the disease when inoculated into monkeys. The adult akamushi collected in Yamagata prefecture by Nagayo gave positive takes in two out of four instances, with only 660 mites, but the 200 we collected in the same prefecture gave no results when injected. These contradictory findings can not be attributed to a difference in the methods of preparation, for we strictly followed Nagayo's technic. It is unfortunate that we can not clear up this discrepancy.

The foregoing experiments clearly indicate that adult akamushi are incapable of infecting experimentally inoculated monkeys. It is very probable that the first-stage nymph would likewise be innocuous, but the larvae which have hatched out from the eggs of adults, carry the virus, although in small quantities, which indicates that it is transmissible by the larval form of the mite alone. This may be explained by the hypothesis that the causative agent is in a phase of development in the body of the adult akamushi, in which it is incapable of producing the disease in monkeys. The fact that the injection of akamushi that have been attached to field voles may cause the disease, can be explained in two ways: It may be due to the virus originally contained in the akamushi themselves, or the virus may be acquired by the mites from the voles by sucking their blood. Both explanations are plausible. It is noteworthy that the virus is very weakly virulent at this time, and the incubation period of the disease is prolonged in cases inoculated through akamushi freshly taken from voles. If the virus be acquired from these animals, it may be that its virulence is decreased at this period, remaining relatively innocuous in the nymph and adult, to be increased in toxicity and regain its original virulence once it

has become located in the mite's ovary. At any rate, the character of the toxicity of akamushi is the same in the natural and the artificial state, but it is contained in greater quantities in the natural and consequently infects the experimental animal more severely and promptly.

5. Immunological Studies.

Kitashima and Miyajima carried out complement fixation tests according to the Wassermann technic, using spleen, liver, or blood of patients, or of infected monkeys for antigen and goats' corpuscles and immune serum for the hemolytic system. They reported that their results were obscure and that no conclusions could be drawn from them.

A. Wassermann Reactions: We carried out Wassermann reactions on eight patients who had no history of syphilis, the tests being done with 0.2 to 0.05 c. c. of serum taken during the fastigium or defervescence; but the results were uniformly negative.

B. Weil-Felix Reaction: This is an agglutination test with Proteus X 19 and was applied to typhus fever by its author in 1915. It is a well-known fact that Proteus X 19 has no relation to typhus, hence the explanation of agglutination is difficult. Kahn and Woithe state that it is a paragglutination. The diagnostic value is, however, certain and almost 100% perfect. There is no question that tsutsugamushi disease is different from typhus, but they have a few similar characteristics in common. Therefore the Weil-Felix reaction might be employed to advantage in this disease also. It has already been tried by Ishiwara and Ogata Jr., who used the serum of three patients during the period of eruption (10, 12 and 17 days after onset) and placental blood of a patient (10 days after onset). Their results were all negative. In some diseases the formation of an agglutinin is slow, therefore it is better to use the convalescent serum. We employed thirteen sera, or the fluid from vesicles, taken during convalescence as well as serum from monkeys after recovery. Six normal sera and those from various forms of disease were used as controls.

The severity of the illness of the patients varied in this series and the duration of the febrile period extended over twelve to twenty-two days. The sera were taken six to sixty-six days after fever subsided. This test is postive in a dilution of x 12,000 in typhus, while in tsutsugamushi disease a dilution of x 50 is the maximum; furthermore, one of the controls also gave agglutination in this dilution. Thus it may be said that the test is negative with the convalescent serum or the contents of vesicles in tsutsugamushi disease, so that it will at least clearly differentiate this from typhus.

CHAPTER V.

ENTOMOLOGICAL CONSIDERATION OF AKAMUSHI.

1. The Prosopon, or Adult Mite.

*A. External Morphology.**

a. General Description.

The prosopon, or adult of the akamushi is a small mite with four pairs of legs which strongly constrict the body between the proterosoma and hysterosoma so as to form a gourd-shaped outline. The body is either light-red or grayish-white. The proterosoma is much elevated, while its anterior half is lower. The hysterosoma is oval, or elliptical in general contour, commonly rounded posteriorly and provided with a pair of longitudinal, parallel and shallow grooves on the dorsum. The ventral surface of the body, unlike the dorsum, has no such distinct furrow separating the proterosoma and hysterosoma. The mouth parts are situated in the fronto-ventral region of the gnathosoma and consists of a collar, the chelicerae, buccal cavity and pedipalpi. The chelicerae are composed of a large buccal segment, usually concealed by the collar which forms the roof of the gnathosoma, and a sharp, strong, spine-like body.

On the ventral surface of the proterosoma are found the two pairs of coxae or epimerae belonging to the first and second pair of legs. The hysterosoma, like the proterosoma, also bears two pairs of legs on its ventral surface. It has two apertures in the mid-line of the venter, the anterior being the genital pore, the posterior the anus. We could not, with certainty, find the stigmata or tracheal orifices which are readily observed at the base of the chelicerae in other trombidiid mites. The adult mites from infected localities measure

* The developmental cycle of this mite was independently discovered in 1914 by Nagayo, in Yamagata prefecture; by Miyajima and Okumura; and by myself and Yamaguchi in Niigata prefecture.

on the average 926.6 μ in length, from the tip of the cheliceral unguis to the posterior end of the hysterosoma; 469.1 μ in breadth anterior to the body-constriction; and 534.0 μ in breadth at the broadest part of the hysterosoma.

b. Integument and Bristles.

i. Integument. This consists of two layers, an inner of living cells constituting the hypoderm, and an outer chitinous cuticle, a product of these. In the male there is a muscular plate, or thick muscular layer 12 μ thick, between the integument of the venter and the gonads, extending anteriorly from the genital pore and posteriorly to the anus. This muscular layer is very poorly developed in the female.

ii. The Bristles or Setae. The entire body of the adult mite is thickly covered with branched bristles, or setae, which are shorter on the proterosoma but gradually become longer more posteriorly. Around their bases are comparatively large sockets. The closed ends of these bristles are pointed in the case of the short ones, but obtuse or clubbed in that of the longer variety. All of the setae are directed parallel and posteriorly, but those near the genital aperture and anus are directed obliquely laterad. Those growing along the metonic crest are apparently twice as long as those of the surrounding areas. The bristles on the coxae of the legs and chelicerae are sharply pointed at their tips. Those covering the pedipalpi and the leg segments, except the coxae, are branched on the external aspect only and are remarkably tapering; while those near the distal extremity of each segment are exceedingly long. On the terminal segments of the first pair of legs the bristles are shorter, more slender, and more compact than on the other parts.

c. The Chelicerae and Collar.

The chelicerae which form the first pair of appendages are situated above the oral opening and buccal cavity. They consist of an immovable paturon and a freely movable unguis. The former is a stout segment of more or less conical shape while the latter is a slightly curved spine-like process, which can be folded back onto the paturon which presents a notch at its ventroapical portion for its reception. The collar, or

dorsal surface of the gnathosoma, first reported in the adult akamushi by Miyajima and Okumura, extends from the anterior margin of the proterosoma to the base of the chelicerae, and is a structure of epistomal nature, serrated at its antero-lateral borders. It bears a single branched bristle.

d. The Buccal Cavity.

The floor of this cavity is prolonged anteriorly in the form of a tongue-like process, which covers in the pharyngeal orifice. The basal portion of the hypostome, where it forms the floor of the buccal cavity and where it abuts on the foramina in the beak, into which the basal segment of the pedipalpi are inserted, is much thickened. The sub-cheliceral plate is a backward extension of the roof of the buccal cavity, to which the dorsal muscles of the pharynx are attached.

e. The Hypostome.

This is a median prolongation of the ventral surface of the gnathosoma; proximally it forms the floor of the buccal cavity and its distal portion is apposed to the ventral surfaces of the chelicerae, distally it extends as the so-called "labium." The labium, chelicerae and hypostome-proper thus form the sucking apparatus.

f. The Pedipalpi.

The second pair of appendages termed pedipalpi or palps, are borne on the antero-lateral angles of the beak, one on either side of the hypostome. Each consists of a more or less cylindrical appendage of five freely-movable segments, of which the second is the longest, the third, fourth, first and fifth following in the order of their length. The fourth segment tapers terminally and bears a series of three slender spines and a single, detached key-seta (or "Tastborste") on its dorsoapical border, with a strong, claw-like appendage at its distal extremity. The fifth, or thumb-like segment, is a soft, clavoid structure articulated through its base with the basoventral portion of the fourth, bearing four key-setae and several branched setae.

g. Ambulatory Appendages.

The legs are of moderate length, composed of six segments (not counting the coxae) and each ends in two sharp and

much curved claws, the inner of which is shorter and more sharply curved than the outer. The claws of the first pair of legs are shorter than the others. These are the strongest legs and their tarsi are stouter and longer than those of the others. The fourth, third, and second pairs follow in the order of their length, the second being the shortest of all. Within the first tarsi are numerous nerve cells and fibers, apparently identical with the "Tastganglien" described by Renking in *Trombidium fuliginosum*.

h. The External Gentialia.

In both sexes these are situated in the mid-ventral line, between the intra-posterior coxal, or epimeral plates and the anus. The genital aperture is a longitudinal slit surrounded by two genital plates.

(a.) Male External Genitalia. These possess two pairs of chitinous genital plates; the external pair together forming an ellipse, have a linear slit between them and, beneath and within themselves, bear three genital suckers on either side. The internal genital plates are covered by a soft integumental layer continued from the base of the genital suckers and serving for the insertion of muscles, the contraction of which would cause them to protrude and function as an intromittent organ or penis.

(b.) Female External Genitalia. The external genitalia of the female are much like those of the male; but the internal genital plates are very minute, poorly chitinized, and lead into the vagina, instead of the ejaculatory duct.

i. Anal Opening.

The anal opening is situated in the mid line at about the middle of the ventral surface of the hysterosoma. The whole structure is elliptical in outline, its main axis being longitudinal. The anus itself is a short longitudinal slit, the margins of which form a pair of stout anal valves. These are semilunar in form and are merely thickened portions of the cuticle, to the internal surface of which the levator muscles of the anal orifice are attached. In cross section the valves appear to project strongly, looking like a beak.

j. The Dorsal Shield.

At the anterior half of the dorsus of the proterosoma, there is the so-called "dorsal shield" or "metonic crest." It is a longitudinal chitinous ridge consisting of three parts, anterior, median, and posterior sclerites; and a single sensory prominence, Berlese's (14) "areola sensilligera." On its outer margin are two pairs of small protuberances, the posterior pair mere elevations, but the anterior bearing deep pseudo-stigmatic apertures from which arise sensory or pseudostigmatic setae. Nerve cells are seen on section, with large and small nuclei, each containing a single nucleolus, lying beneath the chitinous cuticle. They probably represent degenerated eyes, or are related to the sensory setae. The latter are connected with the sensory plate underlying the cuticle and giving origin to nerve fibers.

B. Internal Morphology.

a. The Digestive System.

For the purpose of anatomical and histological description, the digestive system of the prosopon will be conveniently divided into the following successive portions.

i. The Buccal Cavity. The chelicerae and hypostome form a long narrow canal, the buccal canal, which terminates posteriorly behind the base of the hypostome in the true buccal cavity which is much flattened in its horizontal plane and is of considerable width from side to side. The roof and floor of the cavity meet and fuse at its posterior end and along the lateral margins, leaving the anterior portion open to the buccal cavity. The pharyngeal orifice opens into the floor and the only other openings into the buccal cavity are those of the salivary glands, situated at its postero-lateral angles.

The roof of the buccal cavity is continued backward in the form of a broad chitinous plate, the subcheliceral plate, which serves a dual purpose: it forms a smooth surface over which the bases of the chelicerae slide during the process of protrusion and retraction; while the raised, thickened border helps to maintain the chelicerae in position.

ii. The Salivary Glands. These are situated immediately beneath the integument of the sensory prominence and among the muscle bundles of the hypostome, and consist of a single pair of large, glandular masses 50 μ long and 915 μ broad. The salivary duct commences near the posterior limit of these masses and receives numerous secondary ducts which unite with the lobular duct, or with the efferent ducts from one or two alveoli. The main ducts have a diameter of 12.48 μ at their origin in the subcheliceral part of the buccal cavity and, running backward along the side of the brain to its caudal end, attain the approximate width of 18.14 μ. They then course forward and downward, penetrating the brain, and open at last into the floor of the buccal cavity.

Each salivary gland consists of grape-like clusters of alveoli, loosely aggregated into irregular masses. Careful examination shows they consist of three subdivisions presenting different histological characteristics: the first or innermost portion, consists of very large granular cells measuring about 14 μ in diameter, with vesicular cytoplasm, irregularly shaped nucleus, and distinct nucleolus; the second portion occupies the upper, peripheral region and consists of large cells 20 μ in diameter, with a well-stained periphery and a vesicular central portion, each having a centrally located nucleus measuring 4 μ in diameter; while the third portion occupies the lower peripheral region of the gland and also consists of large cells of somewhat pyramidal shape, sometimes as much as 20 μ in diameter, with copious cytoplasm containing large eosinophil granules, and central nuclei of an oval or polyhedral outline.

iii. The Pharynx. The pharynx is that chitinous portion of the alimentary tract which connects the buccal cavity with the esophagus and, in common with that of the majority of the arachnida, it constitutes a powerful pumping organ which sucks in the fluid nutriment and forces it downward into the stomach. Its anterior end is continuous with the buccal cavity near the base of the so-called "buccal spines." The walls are thicker on the upper half of the pharynx and thinner on the lower. From the upper walls radiate two regular series of muscle bands, the sucking and swallowing muscles. The

sucking muscles are attached at their anterior extremities to the chitinous bridge placed between the sub-chelicera! plate and buccal cavity, and at their posterior extremities to the slender tendons arising from the outer aspect of the pharyngeal walls. The swallowing muscles are attached to the dorsal border of the wall of a furrow formed by the fusion of two chitinous walls.

iv. The Esophagus. This is a soft-walled tube of small caliber, which connects the pharynx with the stomach proper. Its course is not quite straight for, immediately at its commencement at the rear of the pharynx, it bends abruptly downward and slightly forward and then, almost immediately, doubles backward forming an S before continuing its course through the brain, which it enters near the antero-ventral surface, penetrating its middle parallel to the ventral surface of the body, and after emerging from it almost directly enters the stomach proper. Near the hind end of the esophagus the walls form a papilla consisting of short, cylindrical cell with small nuclei.

v. The Stomach and its Diverticula. The stomach with its diverticula forms the greater part of the digestive system and is the bulkiest organ in the body. Two large, long, paired, sac-like ceca spring from its anterior portion and run forward; similar, but still larger ceca arise from its posterior portion and run backward. Their general appearance and size depend upon the degree of their engorgement. The empty stomach, with its cecal diverticula, is pale in color but becomes light red when engorged. As digestion proceeds, this changes to gray, owing to the undigested detritus which remains in the lumen. After engorgement, the diverticula become much distended.

The esophageal opening of the stomach is situated at the antero-ventral end, immediately behind the point where this bifurcates to form the anterior diverticula. The wall of the organ consists of two layers: an inner, epithelial layer and a very thin, outer muscular layer, the latter appearing to be imbedded in the delicate homogeneous membrana propria upon which rest the bases of the epithelial cells. Of the epithelial cells, the peripheral are very bulky and transparent,

with minute and irregular nuclei and they contain fat globules, reddish-yellow granules and rarely, black cytoplasmic granules. The muscular layer of the wall does not appear to be as highly developed as in the larva. The muscle bands form an irregular interlacing network which extends over its entire surface, being differentiated into longitudinal and transverse bands with transverse striae.

vi. The Hindgut. This originates above the anterior part of the stomach proper but is separated from it and all efforts to discover a passage between the two on the part of myself and of by collaborator, Dr. Yamaguchi, have been unsuccessful: the hindgut runs upward, then downward, and courses toward the anus after passing over the united portion of the genital ducts. The walls of the hindgut have a membrana propria which supports the flat epithelium, its lumen is filled with black granules.

b. The Genital System.

i. General Consideration. The genital organs of the akamushi are situated in the middle of the body, the gonad being an unpaired organ which lies transversely across its posterior half and leading to the genital duct. In both sexes certain accessory glands are associated with the terminal portion of the latter. The akamushi is oviparous and the spermatozoa are extruded in the form of spermatophores. No special intromittent organ is developed in the male.

ii. Female Genital Organs. The genital organs of the female consist of the following parts:

(1) An ovary:—The appearance and size of this depend upon the degree of individual development. In the material collected in the months of August and September, oögenetic phenomena were frequently observed but the number of ripe ova seen in the ovary was only one or two. In such females the ovary extends from the hind end of the brain to the anus, and from the stomach and its diverticula dorsally, to the walls of the hysterosoma ventrally. It is horseshoe shaped, formed by the fusion of originally paired organs. The lateral extremities and adjacent parts of the ovary are germinal, and more or less developed ova are found near its central portions.

The ova contain each a germinal vesicle, which is rich in chromatin, and have granular cytoplasm. The smallest ovum observed measured 6 μ in diameter, the nucleus about 3 μ, and the nucleolus 2 μ. The largest ova measured 178 μ in length. In fully developed material four large eggs are often found.

2. Two Oviducts:—These arise from the anterior ends of the laterally situated germinal horns, their walls consist of a layer of columnar epithelium with the nuclei near the base of the cells.

(3) A Uterus:—This measures 70 μ x 26 μ, its wall is strongly muscular. The organ makes a conspicuous turn forward and tapers below.

(4) A Vagina:—This opens below, is extraordinarily muscular and lined within by a chitinoid membrane.

iii. Male Genital Organs. These are as follows:

(1) A single Testis:—This is a horse-shoe shaped organ with numerous trifling swellings on the upper and lateral aspects which may be considered the true spermatoblastic area. Within the testis a long canal-like lumen meanders, which may be filled with sperm. Its walls consist of cuboidal epithelium. In a transverse section the testis measures 114 μ x 45.75 μ. Its epithelial cells are very small, but the nuclei are comparatively large and contain each a small nucleolus. The cells measure 12 μ, the nuclei 5 μ. The sperm-cells are found in the testicular lumen, or among testicular cells, and contain each a minute nucleus measuring less than 1 μ. The testis is placed in the middle of the hysterosoma, just the posterior cecal diverticula of the stomach.

(2) Two paired vasa deferentia:—These arise from the horn-like portion of the testis, turn downward and forward and ascend again to enter the seminal vesicle. Their diameter is about 14 μ and their walls consist of columnar epithelial cells.

(3) A single seminal vesicle:—This is median, spheroidal and a very muscular structure measuring 38 μ from side to side and 40 μ dorso-ventrally. The vasa deferentia open from below at its sides.

(4) An Ejaculatory duct:—This commences at the vesicula seminalis and runs downward and backward to the genital aperture, situated ventrally. Its end forms the intromittent organ, or penis, as previously stated.

c. The Nervous System.

The nervous system of the adult mite conforms essentially to the acaridian type, the entire central portion being fused into a single mass surrounding the esophagus, and giving origin to the whole system of peripheral nerves.

i. The Central Nervous System. The brain is a spheroidal mass of nervous tissue situated in the proterosoma and extending from the oral parts anteriorly to the middle of the proterosoma posteriorly, and lying between the metopic crest dorsally and the ventral integument ventrally. It is traversed longitudinally by the esophagus and appears, in consequence, to consist of a supra-esophageal and a sub-esophageal ganglion. These are, however, united on either side of the esophagus by stout connective commissures.

The organ is invested in a closely adherent connective-tissue sheath or neurilemma. The nervous system of the brain consists of a peripheral zone of ganglion cells surrounding a central mass of molecular material, which is traversed in all directions by the nerve fibers forming the various commissures and communicating tracts between the different ganglia. Because of the perfect fusion of the individual ganglia of which the central mass is composed, it is very difficult to recognize them in sections of the brain.

In a specimen of 727.2 μ body length, the brain measures 95.68 μ dorso-ventrally and 74.88 μ along the esophagus; the antero-posterior extension of the supra-esophageal mass measures 79.4 μ and that of the sub-esophageal mass 102.32 μ.

ii. The Peripheral Nervous System. Seven pairs of large nerve trunks are given off from the brain.

(1) The cheliceral nerves, arising from the antero-dorsal extremity of the supra-esophageal mass and running toward the corresponding chelicerae.

(2) The pedipalpal nerves, arising from the antero-lateral corners of the sub-esophageal mass and running to the pedipalpi and mouthparts.

(3 - 6) The four pairs of pedal nerves, springing from the sides of the brain and passing outward one to each leg.

(7) The splanchnic nerves, arising from the postero-lateral angles of the brain and proceeding almost directly backward to the digestive tract, genitalia, and muscular system.

Each nerve-trunk is closely invested by a thin prolongation of the cerebral neurilemma.

d. Sensory Organs.

The sensory organs of the adult that we shall mention are the eyes, key-setae and sensory bristles.

i. The Eyes. These are situated on the sensory prominence, but they are exceedingly degenerated and rudimentary, lacking a lens and represented merely by subcutaneous masses of red granules.

ii. The Key Setae. The key-setae or tactile processes of chitin, are found on the legs, pedipalpi and chelicerae.

iii. Sensory Setae. The sensory setae, or pseudostigmatic bristles, are placed on the sensory prominence of the dorsum of the proterosoma and are finely feathered on their distal halves.

2. The Nymph of Akamushi.

The nymph is very like the prosopon, both in external features and in its internal organization, so that only the distinctive features will be described in the following summary.

A. The nymph is always smaller than the adult, the largest specimen obtained in the field or by artificial breeding was 683.9 μ long, while the smallest obtained from both sources was 401.4 μ long.

B. The bristles covering the body of the nymph are much longer than they are in the adult (see III). Those along the metopic crest of the nymph are shorter and finer than in the adult and number from six to eight on each side. Those on the legs and mouth-parts are fewer, thinner and shorter than in the adult.

C. The tactile spines on the fourth segment, or thumb, of the pedipalpus are two in number, instead of three.

D. The tactile spines on the leg are fewer than in the adult. The key-seta found on the fourth pedipalpal segment in the adult is wanting in the nymph.

E. The genital aperture does not open in the nymph; there are two pairs of genital suckers instead of three. The sexual differences are obscure at this stage.

F. The salivary glands of the nymph are bulky and paired as in the adult, but their constituent cells are uniform in size and shape and are not yet differentiated as in the adult.

G. The gonads of both sexes are already similar in contour to those of the adult, but their tissue is not yet fully developed.

3. Measurements of the Prosopon, Nymph and Larva.

A. Prosopon, Nymph and Larva.

a. The length (in microns).

Stage	Number of individuals examined	Average	Max.	Min.
Prosopon, naturally raised	2	821.1	859.2	789.1
Prosopon, from infected locality	27	926.6	1287.6	701.4
Nymph, naturally raised	8	590.3	666.4	508.5
Nymph, from infected locality	10	491.1	882.9	420.8
Nymph, artifically raised	30	546.0	683.9	455.9
Nymphochrysallis, 4 to 9th day after separation from the ear conch of the field vole	34	403.3	526.1	315.6
Larva, not from field vole	3	252.8	294.0	176.4
Larva, from the field vole	17	364.3	468.0	294.0

b. The width (in microns).

Stage	Number of individuals examined	Average	Max.	Min.
Prosopon, naturally raised	2	410.9	438.4	403.3
Prosopon, from infected locality	27	469.1	701.4	333.3
Nymph, naturally raised	8	362.4	385.8	360.7
Nymph, from infected locality	10	297.4	385.8	210.4
Nymph, artificially raised	30	333.1	333.2	310.4
Nympho-chrysallis, artificially raised	34	284.6	350.7	175.4
Larva, not from vole	3	127.4	147.0	117.6
Larva, from vole	46	216.1	270.0	128.4

B. Different Parts of the Prosopon and Nymph.

Number of individuals examined Prosopon	Average	Max.	Min.	Parts compared	Number Nymph	Average	Max.	Min.
12	287.2	350.7	210.4	Length of pedipalp	8	221.6	288.0	218.4
12	192.3	333.2	105.2	Length of chelium	8	175.4	140.3	140.3
15	66.8	75.5	61.0	Length of cheliceral unguis	18	40.6	52.5	31.96
	29.5	34.8	18.24	Palpal claw	20	22.3	29.5	14.75
	50.2	58.1	43.6	Fourth palpal segment	20	30.2	35.4	23.6
10	17.5	20.3	14.5	Spinelisee body on segment	18	16.3	26.5	11.8
Male 6	61.0	78.4	52.3					
Female 14	119.1	127.8	107.5	Length of genital slit				
	26.6	32.5	17.7	Length of genital sucker	11	17.6	26.5	14.7
	17.7	20.34	14.8	Width of genital sucker	11	12.4	17.7	59.0
26	634.3	806.4	420.8	Length of first leg	35	398.7	473.5	315.6
26	396.6	473.8	298.1	Length of second leg	35	307.1	403.3	192.9
26	377.5	473.5	333.1	Length of third leg	35	307.1	350.7	175.3
26	456.4	613.8	333.1	Length of fourth leg	35	352.0	438.4	227.9
12	72.5	78.5	70.1	Width of first leg	25	47.4	52.6	43.8
12	45.9	52.6	40.3	Width of second leg	25	33.4	35.1	26.3
12	48.3	52.6	40.3	Width of third leg	25	29.5	36.8	26.3
12	48.75	52.6	40.3	Width of fourth leg	25	29.1	35.1	26.3
	27.9			Length of setae of gnathosoma		20.4		
	35.6			Length of setae of Proterosoma		24.39		
	57.2			Length of setae of Hysterosoma		46.16		
				Width of setae of gnathosoma		0.87		
				Width of setae of Proterosoma		1.16		
				Width of setae of Hysterosoma		2.21		
	5.09			Width of setae of body				
15	110.4	136.6	93.0	Length of sensory setae	9	90.1	116.2	72.0
15	40.67	46.5	37.7	Lenghth of setae of collar	12	34.86	43.6	25.9
	14.52			Length of setae of first tarsus				
	23.2	29.05	20.33	Key-seta of cheliceral end		17.43	20.33	14.53

A. The Larva.

A. External Morphology.

a. General Discussion.

When newly hatched the larva measures 210.4 μ, (minimum, 175.6 μ; maximum, 245.5 μ) in length. It is oval in its outline, becoming more eliptical and enlarging after a full feeding, when it measures 352.4 μ on the average (minimum, 210.4 μ; maximum, 473.5 μ). It is bright red or reddish-brown and has six legs.

The body constriction between the second and third pair of legs is not very conspicuous, a transverse furrow rarely exists between the third pair, running over the dorsum of the body. On the dorsal surface of the proterosoma are placed a transverse dorsal shield and a pair of red eyes. The legs have six segments, exclusive of the coxae and each of these ends in three claws. On the postero-lateral side of the first coxa a large spiracle opens, as described by Tanaka. This is often called the protostigma and is slightly elevated above the general surface of the pedal integument. The anal opening is situated at about the middle of the venter of the hysterosoma in the mid line and is elliptical in form, measuring 29.6 μ in length and 22 μ in width, while the slit itself is 4.4 μ wide.

b. The Integument and Bristles.

i. Integument. This is not very firm in consistence and is finely striated, consisting of an outer protective cuticular layer and an underlying hypoderm. The cuticle is thinner than in either prosopon or nymph and measures only 2.9 μ in thickness. The hypoderm is single-layered.

ii. Bristles. Certain modified hypodermal cells of the integument are trichogens, each of which usually retains its connection with a pore in the cuticle, the trichopore, which is continued into the cavity of the bristle it has produced. Almost all of the bristles on the larva are much branched or feathered and their trichopores are surrounded by primary cuticular ring-like elevations which are, in turn, surrounded by finely parallel wavy ridges of the primary chitin. The

number and arrangement of bristles on the pedipalpi and legs are given further on, (see 5 and 6).

The dorsal bristles of the hysterosoma are arranged as follows: 2, 8, 6 or 8, 8 or 10, 8 and a few posterior bristles. The ventral setae are arranged as follows: 2, 8, 4, 4, 6, 6, and marginally 6.

c. The Sucking Organ or so-called "Hypopharynx." This long, tubular organ was first described by Hayashi under the name of "hypopharynx." According to him, it is as long as the body itself, or even one-and-a-half times as long. (e. g. in an individual about 200 μ long, it measures 330 μ) possesses a fine lumen with a thin and readily-stained wall, and expands at its distal end in the form of a trumpet.

We have been able to observe this organ only in a few cases, viz. in two larvae detached from the ear-conchae of field voles and in a third larva found attached to a man. In one of these the structure was 320 μ long and the larva 388.6 μ. In several instances detached symptoms have been observed buried in the integument.

This interesting organ is protruded from between the cheliceral ungues far into the integument of the host, and is without doubt intended for sucking its blood. It measures from 200 μ to 300 μ in length and tapers distally, abruptly to expand at its extremity. It is very resistent to acids and alkalis and practically unstainable with the usual dyes. Around it there is a mantle of a similar chitinous appearance, very resistent to basic dyes, but stainable with hematoxylin and by Van Gieson's method. These suctorial structures are developed after the larva becomes attached to its host and has bitten into it. It is not yet to be seen five hours afer attachment but nine hours after attachment it is already 10 μ long. It was first demonstrated in the European harvest mite by Gudden, who named it the "hypopharynx" and called the surrounding sheath the "Saugrüssel." He considered it to be derived from the tissues of the host, but this view is incorrect and my own observations as well as those of Mukoyama, lead me to conclude that both these structures are derivatives of the larva. The name "sucking organ" is here proposed.

d. The Hypostome and Mouth-Parts. The hypostome is almost quadrate and, in front and below it, appear a pair of cheliceral ungues. The chelicerae are almost concealed by the sac-like membranous extension of the so-called "labium." A laterally extended chitinous carina, present in other trombidia, is absent. The hypostome bears a pair of feathered bristles on its latero-posterior border.

e. The Pedipalpi. These have five segments, stouter in the middle and tapering terminally. The first segment fuses with the hypostome. The bristles on the dorsal surface of the pedipalpus are simple, except that on the fourth segment, which is clearly branched; the lateral and ventral setae on that segment are simple. The terminal claw of the segment is trifurcate and measures about 8.4 μ in length. The fifth segment, or thumb-like body of the pedipalpus is provided with seven branched setae and a blunt tactile key or rod-like bristle.

f. The Ambulatory Appendages. The legs are only six in number, slender and moderately long. The tarsi of the third pair are longer and more slender than those of the rest. The bristles on the legs are markedly branched and the setae on the trochanters are very long and curved. Besides these branched bristles, there are a certain number of unbranched setae on the legs. The patella of the first leg is beset with two plain setae, one dorsal and the other lateral in position; the tibia is armed with two simple setae above; the tarsus is provided with the usual stout, rod-like process and a stiff seta; a very short seta is also found on each of these three segments. The patella of the second leg is armed with a single setae on its dorsum; the tarsus is armed with the usual rod-like process. The patella and tibia of the third leg are armed each with a single seta on the dorsum; the tarsus bears only branched bristles and lacks the long, tactile key seta.

g. The Dorsal Shield. It is situated on the dorsum of the proterosomal region of the body, between the second pair of legs. The scutum is long, but not very wide, with a nearly straight posterior margin. It usually bears seven hairs, which are all very long and slender, the middle one of the anterior margin exceeding the length of the shield in its own length.

The sensory bristles, or pseudostigmatic setae, are inserted nearer the posterior border than to the anterior, with very minute branches limited to their distal halves.

B. Internal Morphology.

a. The Respiratory System. This consists of a pair of spiracles and a system of tracheal tubules.

i. The spiracles. The breathing pores or spiracles are situated on the coxae of the first pair of legs. They are very large and conspicuous and appear as concave pits, measuring 10 μ on the outer, and 5 μ in the inner diameter. From the center an elliptical cleft leads down and opens into a large atrial cavity lying beneath the spiracle and giving rise to the tracheae.

ii. The main tracheal trunks open separtely into the basal portion of the atrial cavity of the spiracle, run downward and inward and then abruptly bend forward and end blindly, measuring 23.6 μ in diameter. From this point two club shaped processes run backward, the outer of these measuring 45 μ in maximum diameter and 9 μ across a point near its base, while the inner one measures 36 μ in maximum diameter and 13 μ in maximum width. These processes are supposed to be the primodia of the air chambers of the respiratory organ.

b. Digestive System. Stomodaeum.

i. Buccal cavity. This is formed by the chelicerae and the oval hypostome.

ii. Salivary Glands. These are very bulky in this larva, unlike those of *Trombidium fuliginosum,* and lie between the inner respiratory organs, the stomach proper and the chelicerae. Their cells measure each from 7 μ to 14 μ in length.

iii. The Pharynx. The pharynx is short and connects the buccal cavity with the esophagus.

iv. The Esophagus. This is a short tube belonging to the foregut, connecting the pharynx and the stomach proper and extending more or less horizontally. The lining of its dorsal and lateral walls is thick, but the esophageal epithelium is greatly reduced.

v. The stomach. There are four paired cecal appendages, running antero-laterally and postero-laterally from the stomach, and each of these presents several smaller, secondary ceca.

vi. The Hindgut. The hindgut extends from above the stomach proper to the anal opening and is filled with minute granules. Its walls consist of a chitinous membrana propria without epithelium. Whether it connects with the stomach or not can not be stated. The granules contained in its lumen are doubly refractive and look black with transmitted light, while they are milky white under reflected light. They undergo no change even after strong heating, are soluble in glycerol after long immersion, and in 1% potassium hydroxide, but not in acids of the same percentage.

c. The Nervous System of the Larva.

This is very similar to that of the prosopon or the nymph. The brain lies above and below the esophagus, which perforates it, and measures 36 μ - 90 μ dorso- ventrally and 54 μ - 108 μ horizontally. The nerves connecting brain and eyes probably radiate from the supra-esophageal ganglionic mass, but they have not been made out definitely. The cheliceral nerves also arise from the same region. From the sub-esophageal ganglia arise the nerves for the pedipalpi, the legs and the hysterosoma.

d. The Sensory Organs.

We shall discuss the eyes, key-seta, and sensory setae.

i. The Eyes. One pair of eyes is situated near the latero-posterior angles of the dorsal shield, on an elevation on either side; they are deep red in life, the pigment being located in the superficial membrane.

ii. The Key-Seta. This organ is found on the tarsi of the first and second pairs of ambulatory appendages, and was discovered by Henking (49) in the case of *Trombidium fuliginosum* and named by him the "Tastborste." He also described a similar organ at the hind end of the body, but this is lacking in akamushi.

iii. The Sensory Setae or Pseudostigmatic Bristles. They are found on the dorsal shield and are described in another section (see 7).

5. The Akamushi and its Allied Species.

Akamushi are found at all seasons within the ear conchae of the Japanese field vole in the localities where tsutsugamushi disease is endemic, not merely from June to October, when the disease is prevalent.

Tanaka (175) was the first to distinguish three kinds of akamushi in 1919, naming them "kedani" and "nezumidani" "a" and "b" on the basis of certain morphological differences. Of their habits he says: "The first kind, or kedani, bites both man and field voles; the second, or nezumidani "a" is, on the contrary, usually parasitic on the vole and only bites man accidentally; while the third, or nezumidani "b" is confined to the vole."

The third type of akamushi can be clearly distinguished from the other two by certain morphological characterisitics, especially by its dorsal shield and the longer spiniform tubercle on the third segment of the pedipalpi. There are, however, no distinct differences between the first and second types of akamushi. Tanaka goes on to say that the "kedani" is a fine-bristled form, but the "nezumidani" has coarse setae; in the former the pseudostigmatic pores on the dorsal shield are placed in front of the origin of the postero-lateral setae, while in the latter they are on, or slightly behind their level; "kedani" are found on the voles from July to September, while "nezumidani" are found on them at all seasons.

Miyajima and Okumura (108), who examined akamushi gathered in different localities, insist that the hair differences between the various forms are due only to seasonal polymorphism, viz. the spring form having stout bristles and the summer form fine ones, but that this characteristic has no taxonomic importance, so that there are no adequate grounds for dividing akamushi into two types.

On the other hand, Nagayo (116) (127) and his collaborators distinguish five types of akamushi, on the basis of morphological and ecological characteristics and are of the opinion that only one of these is the vector of tsutsugamushi disease. They have been carrying on their entomological researches in the infected localities in the prefecture of Yamagata since 1918.

Since then I, too, have been collecting akamushi from within the ear conchae of field voles from the infected localities in the prefecture of Niigata and besides examining them have tried breeding the nymphs and adults from them. Moreover, I have collected adult mites from the ground in endemic localities at various seasons and raised the larvae from their eggs. On the basis of these studies, I recognize the following four kinds of akamushi, exclusive of the Japanese Harvest Mite:

"A" *Trombicula akamushi,* Brumpt.

Common names: "akamushi," proper, A., Yôchû; Kedani; fine-bristled type.

"B" *T. scutellaris,* Nagayo, etc.

Common names: "akamushi B," the "sai-chukan" type.

"C" *T. palpalis,* Nagayo, etc.

common names: "akamushi C," intermediate type.

"D" *T. pallida,* Nagayo, etc.

Common names: "akamushi D," false akamushi, yasodani, the stout-bristled or coarse-haired type.

The only species that I have not been able to find in Niigata prefecture is *T. intermedia,* Nagayo, and c. Of the Japanese harvest mites I recognize two types, E′ and E″. We succeeded in raising the larvae of akamushi A, B, C, D, and E″ from eggs laid in the hatchery by the adults. The larvae of akamushi B, C, and D are similar in size and form to those of A, but they exhibit the following slight differences.

1. A is deep red in color when freshly-hatched from the egg, while B, C, and D are light red.

2. The dorsal shield differs in the degree of roughness of its postero-lateral corners, and in the shape and arrangement of the sensory bristles and the postero-lateral setae.

3. The spine-like process at the venter of the third pedipalpal segment bears a lateral branch in A, while in the others this is lacking.

4. The bristles on the surface of the body differ in the various types.

The Japanese harvest mite E″ corresponds to the Niigata harvest mite of Okumura and to the Akita harvest mite of

Tanaka. Its distinctive character is the strong convexity of the hind border of the dorsal shield and the shortness of its lateral borders, besides having three sensory setae on the tarsi of the third leg and a very long simple on the third tibiae.

The harvest mite E′ corresponds to the European harvest mite of First, described in 1916, and also to the Formosan harvest mite of Miyajima and Okumura; it differs from E″ in having but a single long, unbranched sensory seta on the tarsus of the third legs and in having a long, stalked seta on the dorsal surface of the third segment of the third legs.

6. The Discrimination Between the Various Kinds of Akamushi.

There are two methods employed by Japanese authors to discriminate between the above mentioned types of akamushi; to study the larvae hatched from eggs laid by a given adult and to raise the larvae removed from the ear-conchae of voles through the nymph to the adult stage.

A. Adult Mites.

Two kinds of adult mites have been obtained by us, the first belonging to the genus Trombicula, with a gourd-like body sharply constricted between proterosoma and hysterosoma, the latter being elliptical, or oval, with rounded posterior border; and the second is the harvest mite, with an elongated gourd-like body consisting of broad proterosoma and remarkably slender hysterosoma, producing eggs which give rise to the types designated as E′ and E″. The first type can be subdivided into two subtypes, according to color: red ones and pale red, or white mites; the former being the akamushi proper and the latter including types B, C, and D.

B. The Development of the Larval Mites.

After separating the different sorts of larval mites from the ear-conchae of voles, they were raised in glass vessels. We have been able to trace type A from the larva to the prosopon, through all the intermediate stages; and B, C, D, and E″ from the larva to the teleiophane, through the nym-

phophane and nymph. The nymphs of A, B, C, and D differ in color only, A being red while the others are merely reddish or pale. The nymph of the harvest mite is exactly similar to the adult and differs only in being red; its teleionymph is light red.

The maturation of akamushi varies with the temperature. In the case of Type A, I have been able to raise larvae to the adult form in three weeks in July and August, but five to six weeks are usually necessary.

7. The Relation of the Appearance of Akamushi to Seasons.

The appearance and disappearance of the larvae of the foregoing various types have a certain definite relationship to the season of the year. (Fig. 49). For two years we collected the akamushi attached to field voles and examined them as to their type, the results being as follows.

Type A appears in the middle of June and in large numbers in July, August and September and it disappears at the end of October or the first part of November, not being found during the colder seasons of the year in winter, or spring.

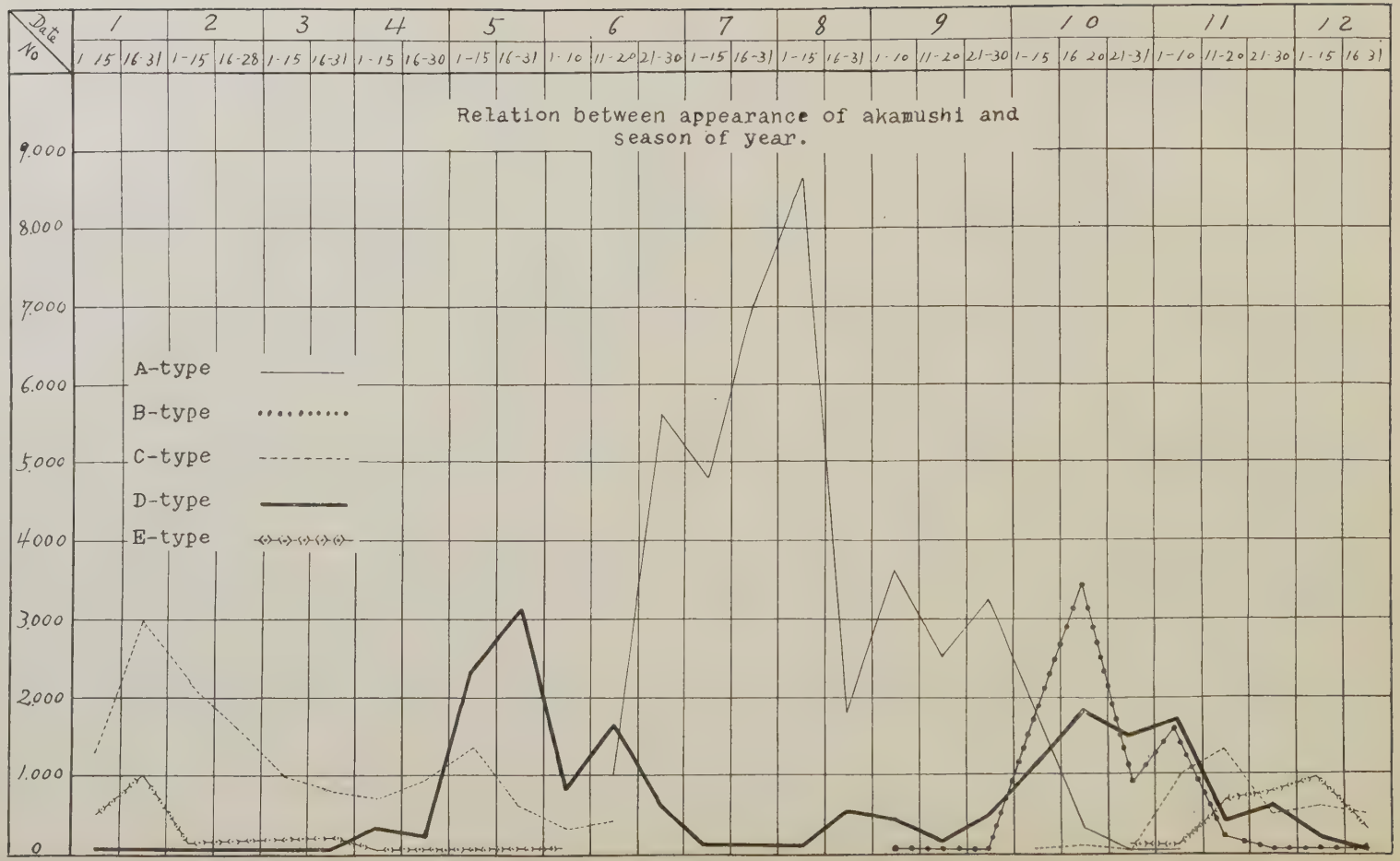
Type B does not appear in the winter or summer, but from September to the end of November.

Type C appears in the first part of October and in great profusion in December, January, February and March. It is not seen during the warm season in June, July, August and September.

Type D appears throughout the year with slight variations; being seen in large numbers in April, May, October and November and decreasing in incidence during the summer and winter.

Type E″ is found at about the same time as Type C, from the end of October to the end of May. Type E′ was seen only in November and December.

These observations are in accord with those of Nagayo, in Yamagata prefecture, but we were unable to find his Trombicula intermedia in Niigata prefecture, as we have already said.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Relation between appearance of akamushi and season of year.
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A-type
B-type
C-type
D-type
E-type

Figure 49

8. Animals Parasitized by Akamushi.

It has already been said that akamushi are found in great masses in the ear conchae of the Japanese field vole *Microtus montobelli.* Life on an animal host is important for the proper development of the mite which could, therefore, be found on other animals than the vole. It may attack warm-blooded animals as it does man, using them for occasional hosts. Thus we may find it, on nature, parasitising the field vole, the Egyptian rat, *(Mus rattus alexandrinus)*; moles, monkeys, rabbits, guinea-pigs, mice, dogs, and cats, when they are kept in the endemic region. Hatori (40) reported in Formosa, that the house rat *M. rattus rufescens;* the sewer rat, *M. decumanus;* the hill rat, *M. jerdoni;* and the field rat, *M. agrestis;* all may harbor the akamushi. He also found insectivorae, *Crocidura muschata* and the shrew *Sorex dzinezumi* infested with it. Among domestic animals, he found it on the dog, cat, and calf. I confirmed this in Formosa in 1920.

Besides these mammalia other warm-blooded animals, such as birds, may harbor the akamushi. Hatori reported that he found them on the pheasant and quail in Formosa and examined a series of wild birds in 1919, finding the true and other akamushi on the pheasant, the turnix (button quail), the kingfisher, goatsucker and cuckoo *(Centropus javanicus).* He found akamushi in the pheasant and button quail; *T. gallinarum* and *T. pseudoakamushi* in the lark-heeled cuckoo; *T. gallinarum* in the quail, and *T. gallinarum* in the goatsucker; but he did not find akamushi on other birds.

Hayashi (43) has reported the result of the examination of birds in an endemic region. Experimenting with ten young quail, he recovered true akamushi from them in two out of three tests. He also caught twelve warblers of the species *Acrocephlus orientalis* in the endemic region and found three of them infested with a few akamushi. In 1920 I realized, while in Formosa, that birds are of great importance epidemiologically and upon my return to Japan, instituted an extensive ornithological survey. From the early part of

August until December men were employed to hunt birds in the endemic region and examine each one taken for attached akamushi.

We examined 412 birds including nine orders and twenty sub-orders. According to Uchida, the authority on the birds of Japan, there are eleven orders in that country, so that there are only two which we have not as yet examined; the procellariiformes (petrels, albatrosses, etc.) and the gruiformes (cranes). Of all the orders of birds examined, akamushi were found infesting the following five: galliformes, (quail, etc.); charadriiformes (plover, gulls, etc.); cuculiformes (cuckoos, parrots, etc.); coraciiformes (owls, goatsuckers, swifts, etc.); and passeriformes (a large order including many families of songbirds: larks, wagtails, fly-catchers, etc.). No akamushi were found on birds of the other four orders: colymbiformes (divers and grebes); anseriformes (geese, ducks, etc.); ciconiformes (waders); and falconiformes (birds of prey).

All those birds upon which akamushi were found were of the type that frequent bushes and shrubs; or, like the quail live on the ground and low bushes. Those that scratch for their food, like the pheasants, present ample opportunity for the akamushi to become attached to them. On the other hand, birds living in the water, or on the wing (like eagles or hawks) afford little opportunity for the mites to attack them. Whether a bird be infested or not depends, then, rather upon its habits than upon its specific characteristics.

As to the types we found. We were able to identify five types (A, B, C, D, and E) on the field vole, monkey, guinea pig and rabbit. The relation of the seasons to the types found have been given elsewhere. The appearance of these types coincides with that of the field voles and other animal hosts. There appears to be no relation between the type of akamushi and the variety of bird which it parasitizes; sparrows and swallows had all the types, turtle doves all but the two E's. The type found on birds depends, then, upon the season at which the bird is taken and various types will be found at various seasons on the same species of bird.

9. Type of Akamushi Biting Man.

It is agreed by several investigators (Tanaka, Nagayo and Hayashi) that type A, which is prevalent in the summer, is the one that bites human subjects; type D also carries the virus of tsutsugamushi disease, as is shown by our experiments, so that a bite of this type would also cause infection. Tanaka has already made this statement. We collected forty-two akamushi that were attached to human beings in various endemic regions of Niigata prefecture and established their types. Thirty-three were of type A, one of which had actually caused the disease; one was of the C-type, and eight of the D-type. Therefore the A-type is the most frequent and while D and C are less important than it is, they are not innocuous and it is possible that the tsutsugamushi disease occurring in late autumn or early winter may be caused by the bite of these types.

CHAPTER VI.

METHODS FOR THE PREVENTION OF TSUTSUGAMUSHI DISEASE.

Ogata recommended the application of a mercury preparation and the internal use of potassium iodide and Hayashi advised the injection of tuberculin as a prophylactic measure, the reason for this being that he noticed while he was experimenting on its transmission that tuberculous monkeys had a somewhat lessened susceptiblity to tsutsugamushi disease. Arguing from this premise, he believed that tubercle bacilli are antagonistic to the virus of tsutsugamushi disease.

Judging from our clinical experience the above preparations, as well as the tuberculin, have no prophylactic value whatsoever. The other method of prevention in vogue, recommended by N. Hayashi, is the widest possible excision of the tissue at the site of the bite. It is reasonable to suppose that if this were removed before the diffusion of the virus an attack of the disease might be prevented, but in practice one encounters several difficulties: one does not know how much tissue to remove, nor how soon it should be excised. In our experience, the removal of enough tissue to cause extensive hemorrhage, twelve hours after the bite was discovered, did not prevent an attack. (See p. 65). Therefore the removal should take place within twelve hours after the patient is bitten. There are very few cases where patients come to a physician within so short a time and many of them will not even notice that they have been bitten until the disease develops. As to the extent of the excised area: according to our histological examinations, the necrosis and cellular infiltration penetrate deeply into the subcutaneous tissue, wherefore a superficial removal will not suffice and it will be seen that reliance on this measure alone is unsafe. We must devise other methods: the akamushi should be exterminated in the endemic regions and we must render them incapable of attacking the human body.

1. Experiments on the Extermination of Akamushi.

Much experience has taught us that the building of embankments to prevent floods, and the reclamation of waste land will gradually exterminate the akamushi. Another method serving the same purpose is to exterminate the field voles in the endemic regions, for akamushi usually live in large colonies in their ear conchae and by exterminating these hosts with phosphorous, arsenic, or by inoculation with the bacillus of mouse-typhoid, the terrain for breeding akamushi can be greatly reduced. As voles also damage farm produce, we should be killing two birds at one shot by exterminating these pests. These measures have been extensively carried out in Niigata prefecture for over ten years, but the results have been discouraging, the number of cases of tsutsugamushi disease not being materially reduced thereby. It has been thought that the reason for this failure was that the field voles are not the only hosts, as there are many birds which harbor the mites and the extermination of the voles alone would therefore be insufficient.

The best method, therefore, is to attack all forms of akamushi. As it affords great possibilities we have focussed our attention chiefly upon this aspect of the problem, studying the effect of various conditions and chemicals upon these mites.

A. Experiments Upon the Resistance of Akamushi to Various Stimuli.

The mites we used were either hatched out in an incubator or collected from the ears of voles.

According to the results here shown, a temperature of 30° - 35° is the most suitable for the growth of akamushi. Whether on land or in the water, it moves most actively at this temperature, which agrees with the fact that akamushi bite the most during the warm summer season. They may move about only for an hour at a temperature of 0°, which demonstrates that the mite can not live long in the winter unless sheltered in the ear of the field vole. It lives less vigorously in direct, hot, summer sunlight than in a shaded room and survives for about a week while floating upon the surface of water in a room.

As to the action of chemicals: 5% phenol or mercuric chlorid kills akamushi in a few minutes, 1% mercuric chlorid kills it in one to two hours and 0.1% is not fatal for at least three. Akamushi move about actively in 70% alcohol for twenty minutes, gradually becoming motionless after thirty minutes, but pure alcohol kills them almost immediately, while 4% formalin also kills at once, its vapor being fatal in a few seconds. 1% cresol is fatal in a few seconds and is more efficacious than phenol or mercuric chlorid, and solutions of cresol-soap are also very effective, killing almost instantaneously. 10% potash soap is weaker, being fatal in ten minutes. Crude petroleum and refined petroleum have almost the same effect as this. Camphor in pure alcohol kills immediately, oil of camphor in a few seconds. The vapor of naphthalin kills in two to three minutes, and the fumes of burning sulphur in one to two seconds. The "vermifuge chrysanthemum" has no action on the mites. 10% disinfectol ("Desin") kills them in about two minutes and 50% in one.

Akamushi are comparatively resistant to various acids; 5% solutions of sulphuric, nitric, hydrochloric or acetic acids do not kill for at least one or two hours, while concentrated solutions are immediately fatal. They are also comparatively resistant to alkalis, living in 1% sodium hydroxid and surviving in 1% ammonia for over twenty-four hours. A 1% solution of mint will kill the mite in two minutes, pure mint immediately, and its vapors in one to five minutes.

In the practical application of these experiments, it is shown that petroleum, particularly the crude product, is the best material to use for the extermination of akamushi over large areas. Lysol is the best medium for killing them when attached to humans, a 1% solution proving fatal in a few seconds. The next best remedies are: 5% phenol, and then pure ether, chloroform, and creolin. A spray of mint is also effective. The akamushi may be removed, if they have not yet bitten, by immersing one's body in water.

After people enter the endemic region akamushi may be found in their clothes and may bite any one handling them, so that disinfection of the clothing is very important. Of course, boiling or steaming will kill the mites immediately,

but as this is sometimes impracticable formaldehyde or sulphur dioxid may be used advantageously.

B. Experiments on the Resistance of Adult Akamushi.

In order to exterminate akamushi, it is important to kill off the adults. We collected several thousand of these and experimented with different germicides as to the time required to cause cessation of motion. The results demonstrate that the most effective substances are cresol-soap (lysol), phenol, neosol, and "Desin"; in comparatively high dilutions; while sulphur dioxid and iodine vapor are also effective as fumigants. Among the various mineral oils, the preparation of the Hoden Oil Co., "A," proved the best, the mites being killed in from 10 - 20 seconds in the undiluted oil and in 7 - 15 minutes in the oil diluted x 100.

C. Experiments on the Resistance of Larval Akamushi to Chemicals.

In the preceding experiment we ascertained those preparations which had the most efficacy in killing adult akamushi, we next carried out a similar series of tests with these substances on larval mites collected from the ears of field voles or hatched out in the incubator.

This experiment demonstrates that neosol is the most effective killing vole-bred larvae in 20 - 30 seconds in concentrated form and 40 seconds to 2 minutes, (15 seconds in incubator larvae) in a dilution of x 100. The next most efficacious chemicals are: Cresol, phenol, and oil; "Desin" being the least effective in its action.

D. The Cost of Disinfective Materials.

The practical application of a disinfectant over a wide area depends largely on its cost, if this be prohibitive the most effective preparation is impracticable for use. A pound of neosal costs 2.30 yen ($1.15), while one of "Preparation A'" of the Hoden Oil Co., costs only 13 sen (6c), and it may be bought in large quantities. Therefore we decided to use this in the practical application for the disinfection in the endemic regions. As a dilution of a hundred times of this

material is less effective against adult akamushi than it is against their larvae, it was necessary to ascertain the optimum dilution with an admixture of neosol so we carried out experiments on artificially infested soil.

E. Experiments on Artificially Infested Soil.

Early in June we collected earth from an endemic region and placed it in a glass vessel of 1-150th. tsubo (7 cu. ft.) capacity, spreading it to a depth of 8 inches, and half-burying the vessels in the ground with their edges projecting above it. Into each vessel we put 100 active adult akamushi and sprayed various solutions over the earth three days later, in order to determine their comparative effectiveness.

We have found akamushi buried seven or eight inches below the surface of the ground in winter, with the advent of warm weather they gradually approach the surface and in summer live near it, being found on it in the morning and evening, or in shady places. Therefore, if the spray permeates the earth to a depth of two or three inches, it should act upon the mites. We found our solution permeating the earth in the jars to a depth of two or three inches.

In every case, two to twenty hours after spraying, the earth was removed and the mites examined, the majority of them being found in the upper two inches and only a few deeper than this. As the table shows, the most efficacious solution was the petroleum emulsion in a dilution of x 100. Upon examining the earth three-and-a-half hours after spraying, only eleven akamushi were found alive and five survived after being placed in water over night, while eighty-four were found dead. This gave us a mortality of 84%. In all cases those mites that survived the spraying were found below the two inch level.

This experiment indicates that the larval form may also be effectively exterminated by spraying petroleum emulsion, for they are also found near the surface during the summer season and are less resistant to the spray than are the adults. Consequently I believe that the akamushi will be completely exterminated by spraying infested ground several times. This solution is very inexpensive, which is requisite for its use over large areas of territory.

F. Experiments on Preventing the Attachment of Akamushi.

The next point in the prevention of tsutsugamushi disease is to devise a method whereby akamushi may be prevented from attaching themselves to their victims. For this purpose various solutions were applied to monkeys, rabbits and guinea pigs and these were exposed in endemic regions to test the protective effect of these preparations.

a. Experiments with Ointments. When certain animals: monkeys, rabbits, guinea pigs or rats are exposed in the endemic region, akamushi attach themselves to the ears, eyelids, nose, face, scalp, and the belly. The mites attack monkeys and guinea pigs more particularly. We prepared ointments containing various percentages of neosol "Desin," oil of camphor and naphthalin and applied these to the parts most likely to be attacked by the mites, and then exposed the animals in endemic regions.

i. Neosol Ointment. The results in monkeys and rabbits to which the ointment was applied were not clean-cut, as only a few akamushi were found on both experimental and control animals. But with the guinea pig the results were very definite. When an ointment containing over 10% of neosol was applied there were very few akamushi as compared with the controls, which were not anointed.

ii. "Desin" Ointment. With 5% "Desin" ointment there was a somewhat smaller number of akamushi on the experimental animals than on the controls, but with an increase in the percentage of "Desin" the attachment of the mites became less frequent, 20% ointment almost completely preventing it. The results with this preparation were better than those with neosol.

iii. Oil of Camphor Ointment. The application of 5%, 10%, and 20% oil of camphor ointment completely prevents the attachment of akamushi.

iv. Naphthaline Ointment. The application of naphthalin ointment in percentages of 5, 10 or 20 had no effect whatever on the mites.

This experiment shows that the best ointment was that containing oil of camphor, which prevented the attachment of the mites in 5% concentration and the next most effective was "Desin," which prevented attachment in percentages of 10 or 20. Probably their action is not due to the germicidal effect of these ointments, but rather to their volatile gases or their odor. "Desin" and oil of camphor are made from crude oil of camphor, having a very pungent smell. Application of these ointments to the human body presents some difficulties, hence easier methods must be found and for this reason we experimented with sprays of neosol and "Desin."

b. Experiments with Sprays. For various reasons we used neosol and "Desin," both of which can be highly diluted. Animals were sprayed with them three times a day and exposed in endemic regions. This experiment was done during September and October, when there were frequent rains which tended to wash off the substances from the bodies of our experimental animals; therefore the weather was carefully noted.

i. "Neosol." The experiments in which dilutions of one to 100 and one to 200 of neosol were sprayed over guinea pigs, monkeys and rabbits demonstrate that the results were equally good. On rainy days, however, they were unsatisfactory on account of the neosol being washed off the animals.

ii. "Desin" Spray. Experiments with "Desin" in dilutions of 100 and 200 were carried out by spraying guinea pigs and monkeys. The results were remarkably satisfactory, during fifty or sixty experiments no akamushi was found on the guinea pigs and only twice were they found on monkeys.

A comparison of the ointment with the spray-methods shows that "Desin" gives equally satisfactory results, while neosol is better as a spray than as an ointment. The spray-method is not only more effective than ointments, but it is likewise more convenient and can be easily applied. In the practical application "Desin" is cheaper than neosol, but it stains the clothing, therefore neosol is preferable.

2. The Practical Application of Preventative Measures Against Akamushi.

In carrying out various experiments to prevent akamushi from attaching themselves to the human body we had two immediate objects: to prevent tsutsugamushi disease among the laborers working on an extensive engineering project along the banks of the Akano River, where many died every summer from this disease and to protect our collectors of material for the investigation of tsutsugamushi disease against the bites of the akamushi. The application of these measures was varied to fit circumstances.

A. Methods of Application along the Akano River.

One of the most dangerous tsutsugamushi disease regions in Japan lies along the Akano River in Niigata prefecture, where this malady takes a toll of from fifty to sixty lives every

year. The yearly inundations, however, make this soil very rich so that this is the center of rice cultivation. The Department for Home Affairs undertook to check the floods by building embankments and repairing the river bed and set hundreds of laborers to work. It became an urgent necessity to exterminate the akamushi in order to protect these men from tsutsugamushi disease and the Government asked our aid in combatting this difficult problem. We, on our part, carried out experiments to determine the most feasible methods for so doing, as already described.

a. Extermination of akamushi in the ground: As we found that the Hoden Oil Co. Emulsion A' was the most effective medium for exterminating akamushi in the ground, we decided to use it in practice, in the dilution of one to one-hundred. A 45-gallon spray-pump was built and the emulsion was slowly diluted with one-hundred parts of water, the ground was well wetted-down with water and sprayed with the emulsion.

b. For the protection of the body the men were sprayed with 1:100 "Desin" or neosol three times a day.

c. Since spraying the body with these was not absolutely certain to protect men working on unsprayed ground, we devised a protective suit of four pieces, as shown in Fig. 50 which was put on before its wearer entered unsprayed territory and was fumigated as soon as taken off in the evening, with formaldehyde, which kills the akamushi almost instantaneously. This suit covers the entire body excepting the eyes.

B. Practical Results in the Akano River District.

We applied the above described preventative measures throughout the summers of 1919, 1921, and the following years to date, with the result that the men working in the treated area were not bitten by the akamushi and did not contract tsutsugamushi disease during all this time; whereas, in the surrounding villages, a considerable number of farmers were bitten, several of them contracting the disease. This remarkable result facilitated the work of the Government and it progressed rapidly.

C. *Results Among Our Collectors.*

We have been employing several men since 1918, to collect various materials for our investigation of this disease and this was a very dangerous occupation for them, as they had to enter the endemic region to catch voles and other infested animals, for other reasons connected with the investigations. We supplied them with the protective suits just described and with a small spray outfit and a solution of 1:50 "Desin" to be repeatedly sprayed over their bodies in the course of a day. They entered the most thickly-infested regions almost every day during the summer for several years, but not once were they bitten by akamushi or did they contract tsutsugamushi disease, which shows the efficacy of our protective measures.

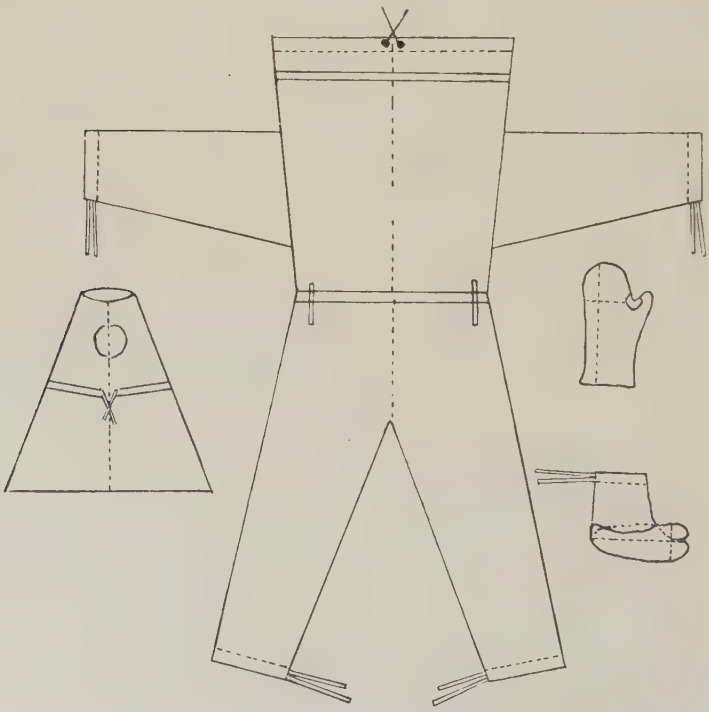

Figure 50
Germ-proof Clothing

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EXPLANATION OF PLATE FIGURES.

PLATE I.

Fig. 1. View of an endemic locality on the Shinano River.

Fig. 2. A bite-lesion in the left axilla of a boy of 15.

PLATE II.

Fig. 3. A bite-lesion near the outer canthus of the right eye and swelling of the preauricular nodes in a man of 20.

Fig. 4. An incrusted bite-lesion on the posterior surface of the leg of a woman of 23.

Fig. 5. An ulcerated bite lesion in the left groin of a woman of 35.

PLATE III.

Fig. 6. Photomicrograph of a bite lesion excised on the fourth day after onset of symptoms in a man of 34. Limited necrosis.

Fig. 7. Photomicrograph of a bite lesion excised one week after onset of symptoms in a man of 70. Deep necrosis.

PLATE IV.

Fig. 8. Photomicrograph of a bite lesion excised on the fifth day after onset of symptoms in a man 51. Superficial crust formation.

Fig. 9. Photomicrograph of a field from Fig. 8 highly magnified.

PLATE V.

Fig. 10. Photomicrograph of a bite-lesion excised from a man of 57, who died about a week after onset. There is a hard crust distinctly adherent to the underlying tissue. Karyorrhexis in exudate indicated.

Fig. 11. The same under higher magnification, to show high degree of karyorrhexis in nuclei of exudate.

PLATE VI.

Fig. 12. Photomicrograph of an atypical bite lesion from a man of 34.

Fig. 13. Photomicrograph of a bite lesion from a reinfected patient; woman of 27.

Fig. 14. Higher magnification of a field in the deeper portion of Fig. 13.

PLATE VII.

Fig. 15. Photomicrograph of a primary bubo from a man of 57. A focus of necrosis beneath the infiltrated capsule of the node and some medullary infiltration.

Fig. 16. Higher magnification of lymphoid tissue adjacent to a necrotic area. The large phagocytes, mingled with lymphocytes, are very evident. Note the pycnotic nuclei they contain.

PLATE VIII.

Fig. 17. Photomicrograph of a primary bubo from a fatal case in a man of 57; Weigert's stain. The lymph sinus is converted into a ribbon-like band of necrosis, other necrotic areas are disseminated through the follicular cords. Note the deposition of fibrin.

Fig. 18. A secondary bubo (mesenteric lymphnode) from a fatal case in a man of 42 who died 11 days after onset. There is proliferation and necrosis of cells.

PLATE IX.

Fig. 19. A primary bubo excised from a man of 34 four days after onset. Cellular proliferation without necrosis.

Fig. 20. A greater magnification of a field from preceding section. Many large vesicular cells mingled with the lymphocytes and plasma cells.

PLATE X.

Fig. 21. Photograph of the eruption in a woman of 22, taken six days after onset.

Fig. 22. Eruption on the arm of a man of 22, one week after onset of the disease.

Fig. 23. The dark mass in the ear of the vole is a collection of akamushi.

PLATE XI.

Fig. 24. Sketch of eruption on the arm of a man of 30.

PLATE XII.

Fig. 25. Sketch of the enanthem, or eruption on the soft palate and roof of mouth, from the same man.

PLATE XIII.

Fig. 26. Photomicrograph of a papule in the eruption from a woman of 46.

Fig. 27. Magnified field from the field marked with a cross in Fig. 26.

PLATE XIV.

Fig. 28. Photomicrograph of a papule from the eruption in a girl of 13.

Fig. 29. Photomicrograph of vesicular papule, the epidermis is converted into a series of multilocular cysts by the colliquation necrosis. From a boy of 19.

PLATE XV.

Fig. 30. A focal necrosis in the liver of a man of 57.

Fig. 31. Focal necrosis in the spleen of a man of 42.

Fig. 32. Focal necrosis in the marrow of the same case.

PLATE XVI.

Fig. 33. Photomicrograph of a bite-lesion with the akamushi in situ. Human case, sucking organ evident.

Fig. 34. A similar photomicrograph from a monkey's skin.

Fig. 35. Human case; there is so much cellular infiltration near the bite wound that this may be considered a bite of an infective akamushi, or a primary lesion of the disease.

PLATE XVII.

Fig. 36. To show the sucking organ of the akamushi about five hours after an artificially induced bite.

Fig. 37. The sucking organ 24 hours after such a bite. The organ is much more evident and nodal constrictions may be seen at intervals along its wall.

Fig. 38. Perfectly developed sucking organs of two mites, inserted into the tissue on the inner side of the ear concha of a field-vole.

Fig. 39. The sucking organ inserted into the tissue of a fowl. It is surrounded by a firm, homogeneous sheath, with a linear area of softening between this and the syphon.

Fig. 40. The inner side of the concha of a vole, showing numerous sucking organs of akamushi out in various planes of section.

PLATE XVIII.

(Various Typfs of Akamushi).

Fig. 41. A- or fine bristled type.

Fig. 42. The same engorged.

Fig. 43. B- or "sai-chukan" type.

Fig. 44. C- or intermediate type.

Fig. 45. D- or coarse bristled type.

Fig. 46. E- or Japanese Leptus autumnalis type E".

PLATE XIX.

(Scutum of Akamushi).

Fig. 47. Scutum of intermediate type, C.

Fig. 48. Scutum of "sai-chukan" type, B.

Fig. 49. Scutum of coarse bristled type, D.

Fig. 50. Scutum of fine-bristled type, A. (True akamushi).

PLATE XX.

Fig. 51. Nymph of type-A.

Fig. 52. Adult, or prosopon, of type-A.

Fig. 53. Second stage pupa.

Fig. 54. First stage pupa.

Fig. 55. The scutum of the adult.

PLATE XXI.

Fig. 56. Adult Japanese Leptus autumnalis, (Type E")

Fig. 57. Nymph of same.

Fig. 58. Scutum of L. autumnalis (Type E')

Fig. 59. Scutum of L. autumnalis (Type E")

Fig. 60. One erect, hard bristle on tibia of Type E'.

Fig. 61. Four erect, hard bristles on that of Type E".

PLATE XXII.

Tissue Sections of Adult Akamushi, Transversely Cut.

Fig. 62. Sp. salivary gland; Gn. ganglion; L. M. liver-stomach.

Fig. 63. Sp. salivary gland; Gn. ganglion; L. M. liver-stomach, Os. esophagus.

Fig. 64. Transverse section of female. Ov. ovary; L. M. Liver-stomach; Ed. hindgut.

Fig. 65. Cross-section male. Hd. testis; Br. intertesticular bridge. Mp. muscle plate; Ed. hindgut.

PLATE XXIII.

TISSUE SECTIONS OF AKAMUSHI AND ITS ADULT.

Fig. 66. Longitudinal section of larva. Legends the same.

Fig. 67. Another longitudinal section.

Fig. 68. Longitudinal section of adult. Gn. ganglion; Mal. malpighian duct; L. M. liver-stomach; Hd. testis; Br. intertesticular bridge.

Fig. 69. Transverse section of the south parts of the adult. Ch. chelicerae; Mt. maxilla; IB. frst leg; IIB. second leg; Ch. chitinous bridge; Ph. pharynx; L. cleft between chelicerae and chitinous bridge.

PLATE XXIV.

Fig. 70. Engineering project in the disinfected region.

Fig. 71. Disinfection of infested regions with petroleum emulsion.

PLATE XXV.

Fig. 72. Men catching field-voles while protected by the vermin-proof suits.

Fig. 73. Spraying with neosol a coolie entering the infested area.

Fig. 74. A man clad in the vermin-proof suit.

PLATE I

FIG. 3

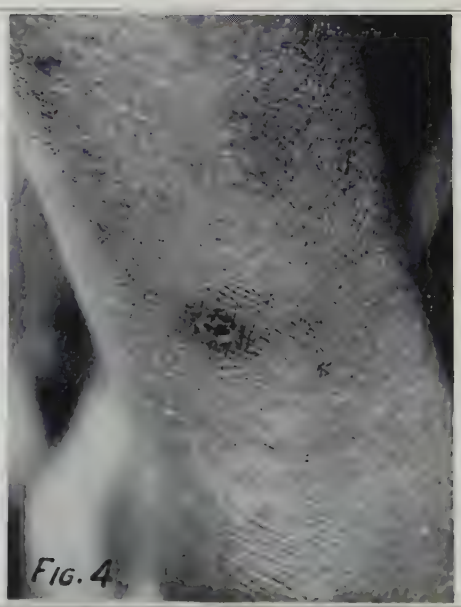


FIG. 4

FIG. 5

PLATE II

FIG. 6

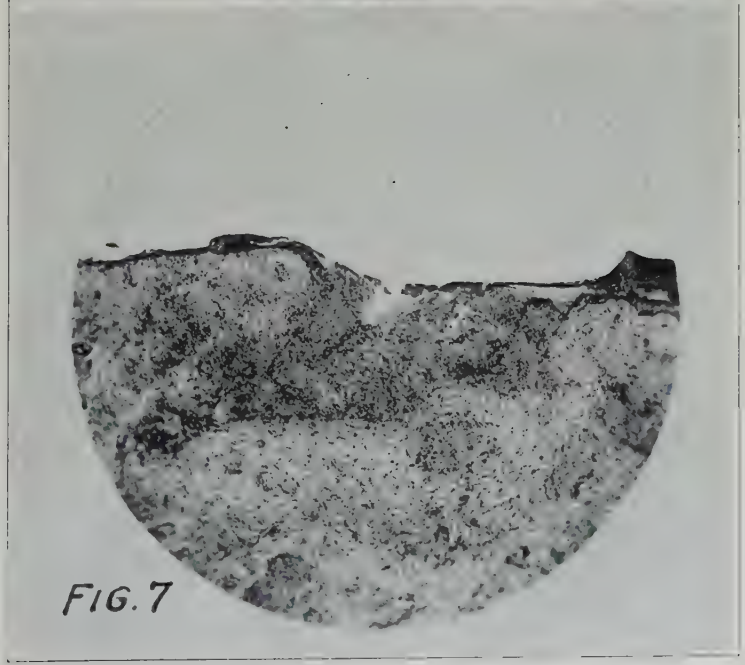


FIG. 7

PLATE III

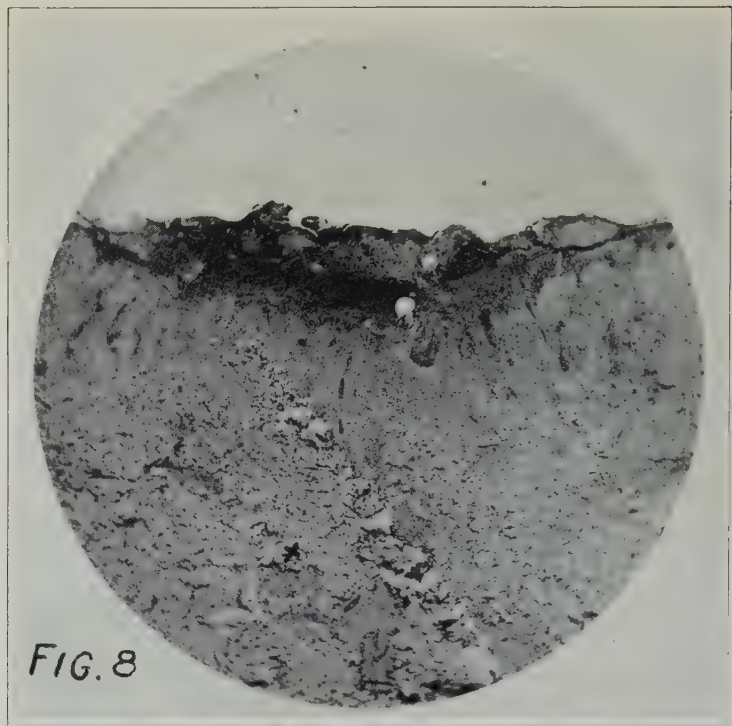


FIG. 8

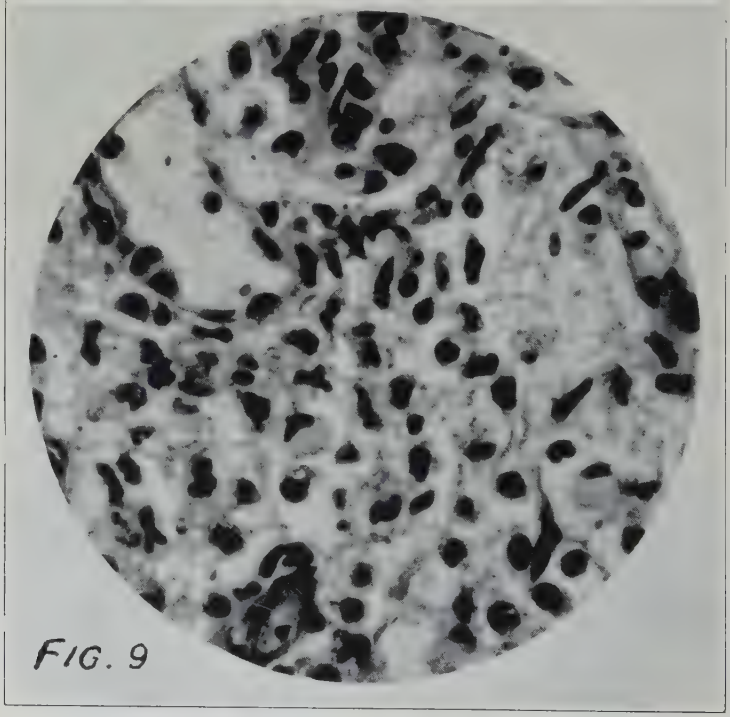


FIG. 9

PLATE IV

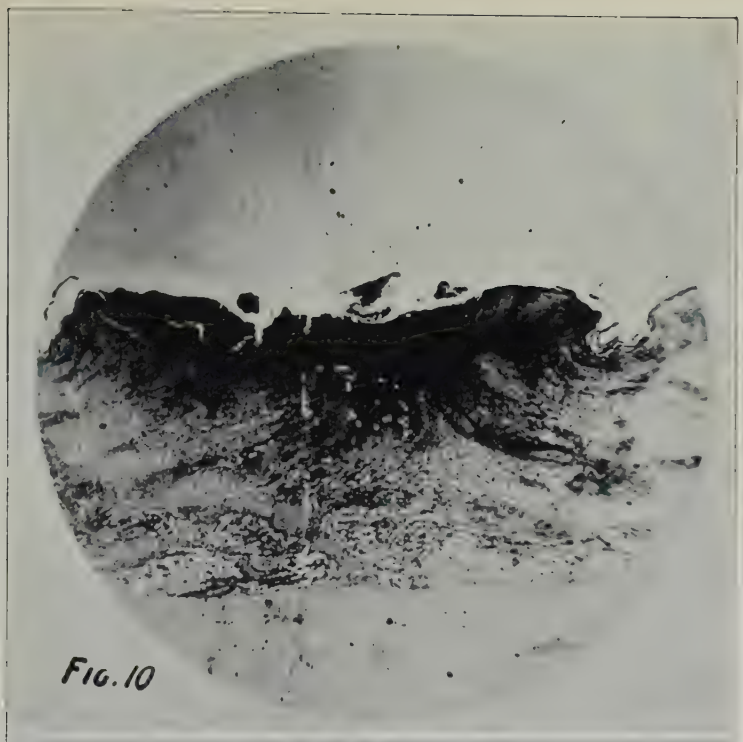


FIG. 10

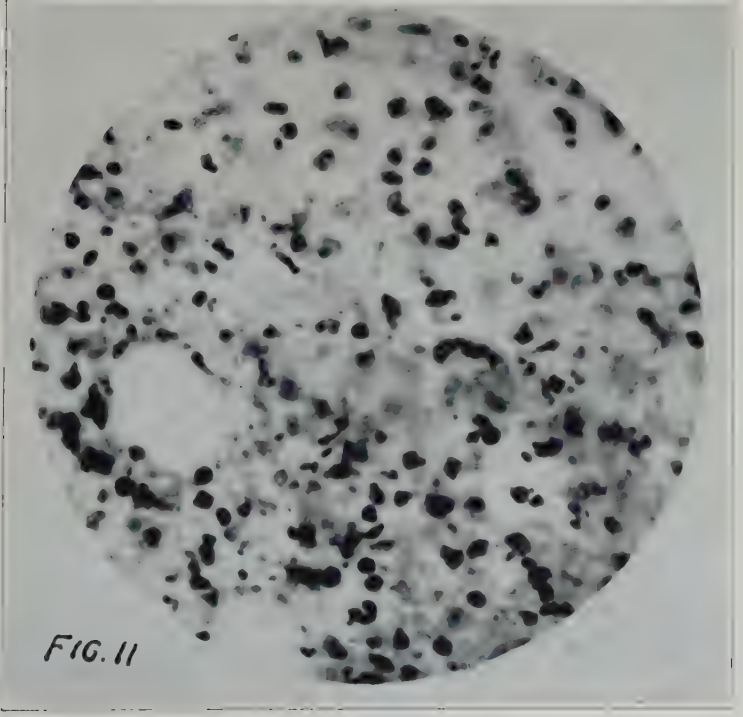


FIG. 11

PLATE V

FIG. 12

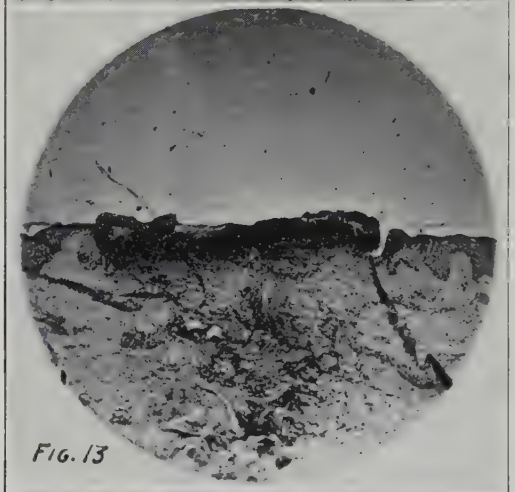


FIG. 13

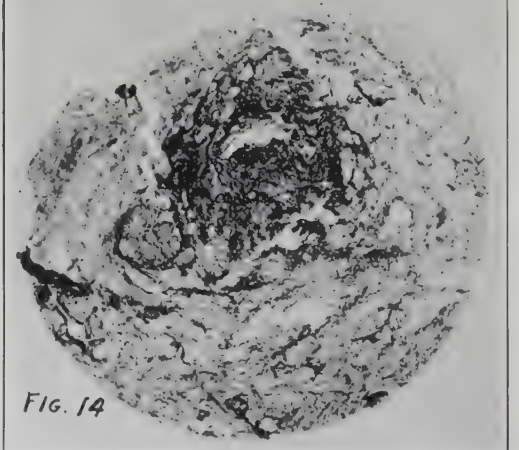
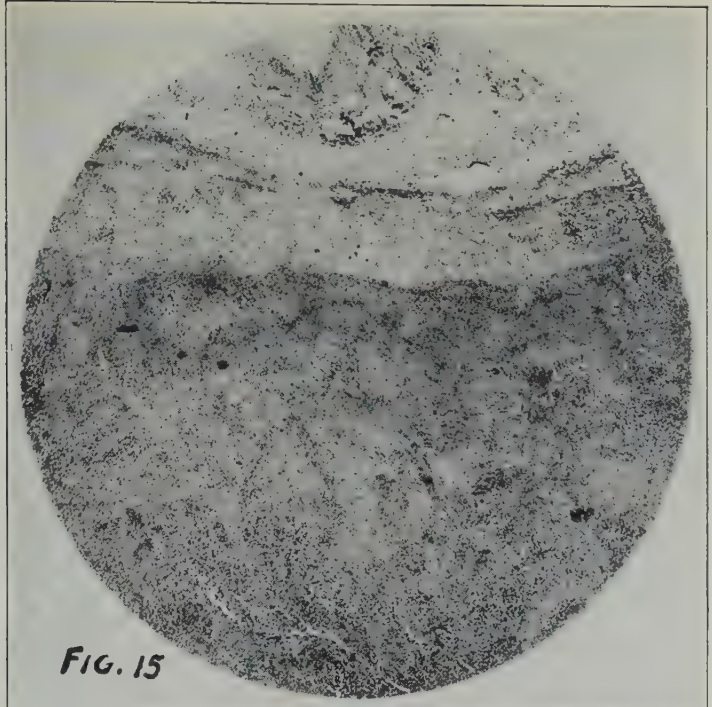


FIG. 14

PLATE VI



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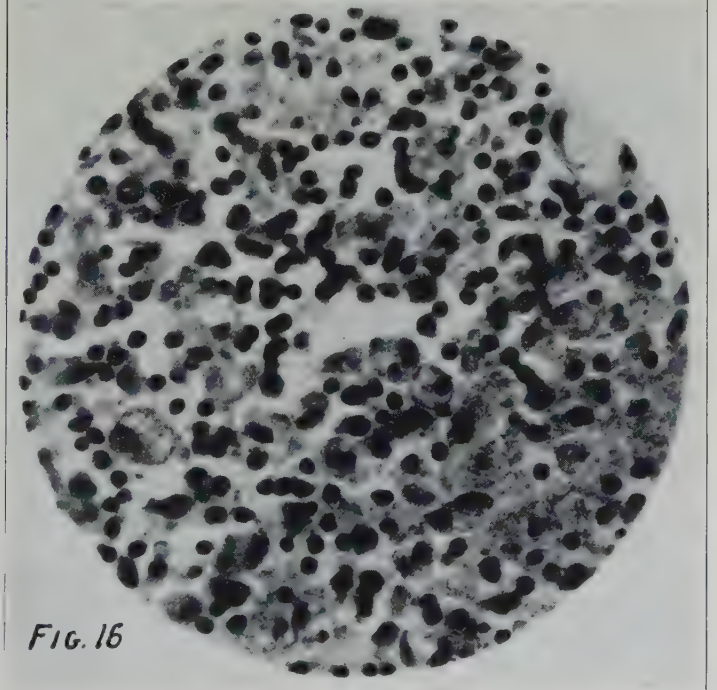


FIG. 16

PLATE VII

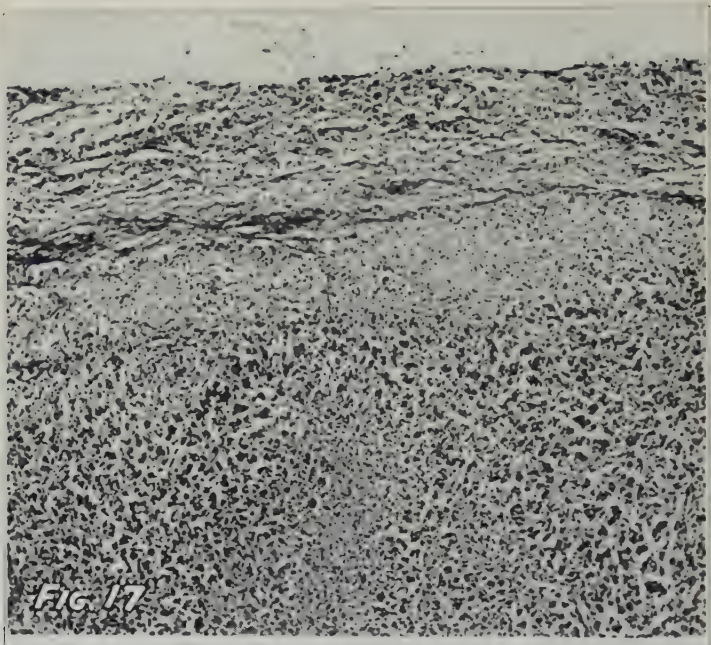
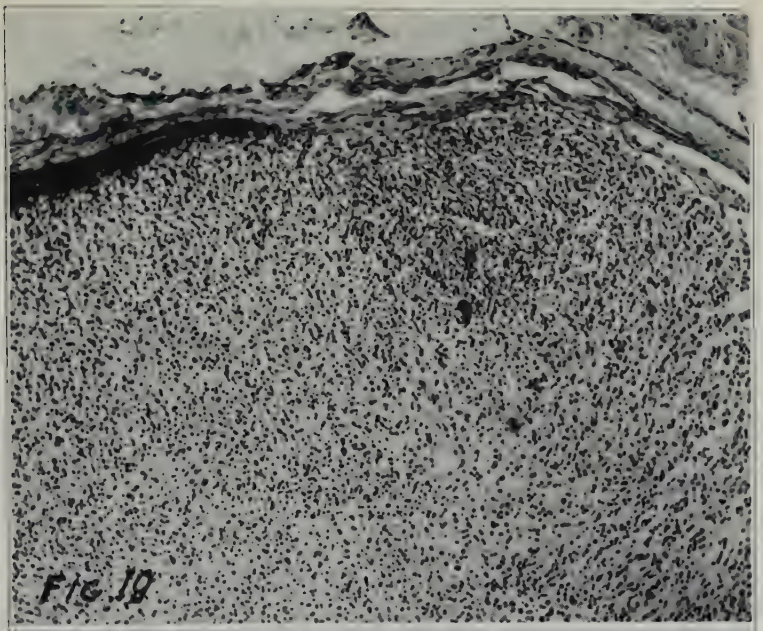


PLATE VIII



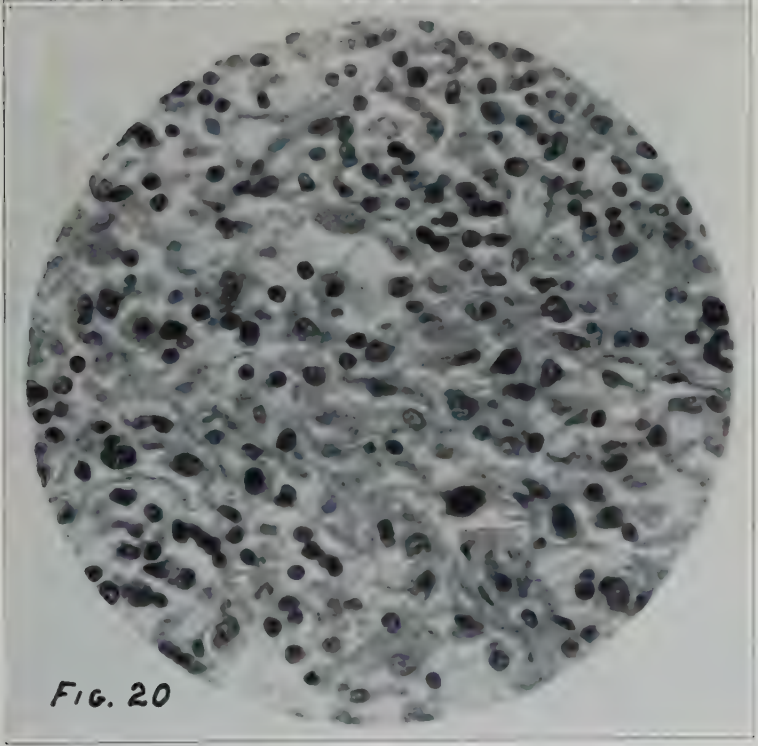


PLATE IX

PLATE X

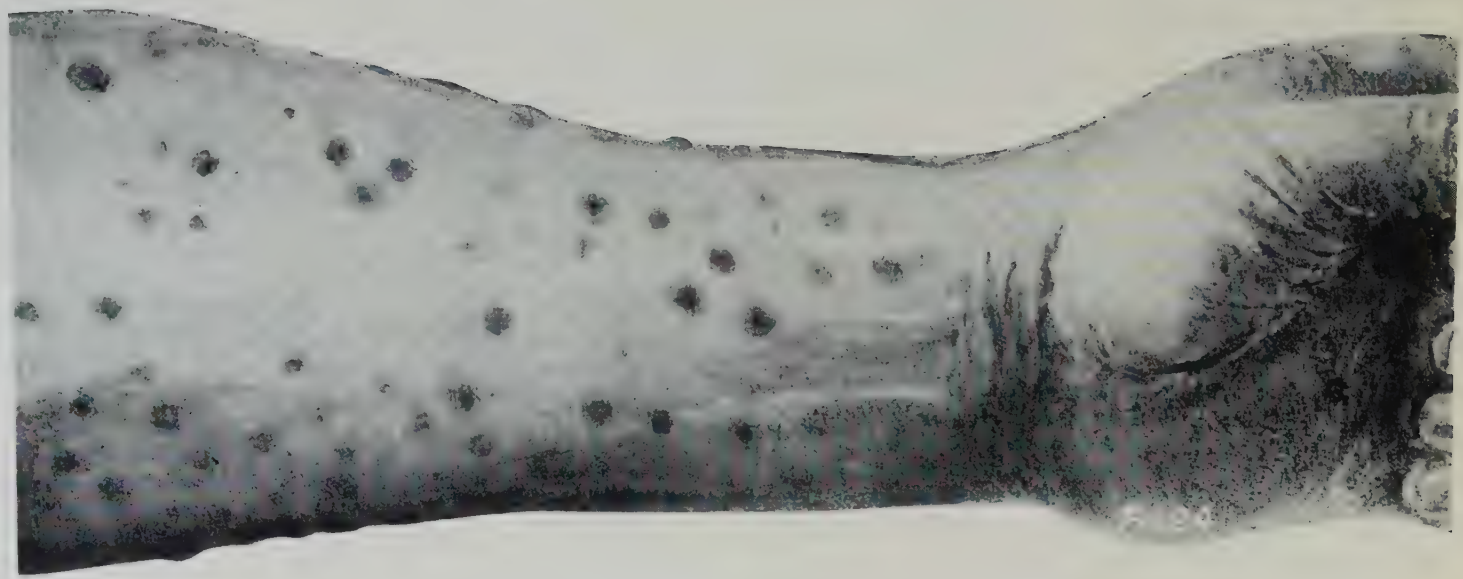


PLATE XI

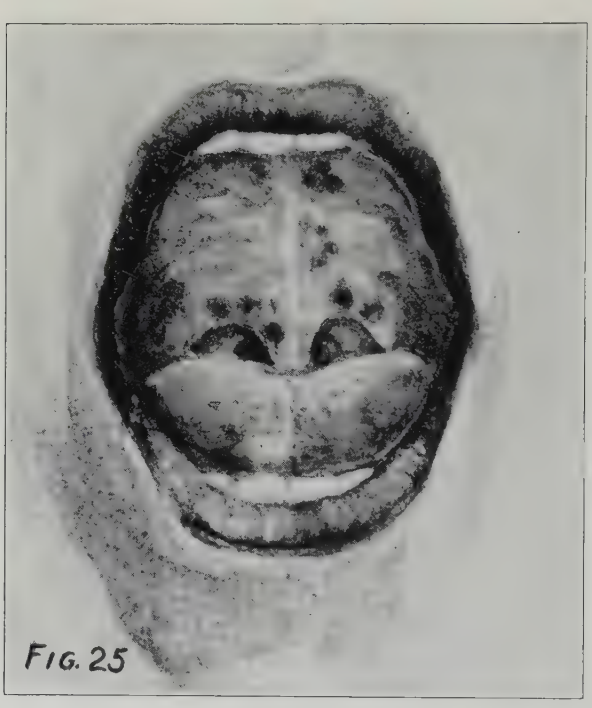


FIG. 25

PLATE XII

FIG. 26

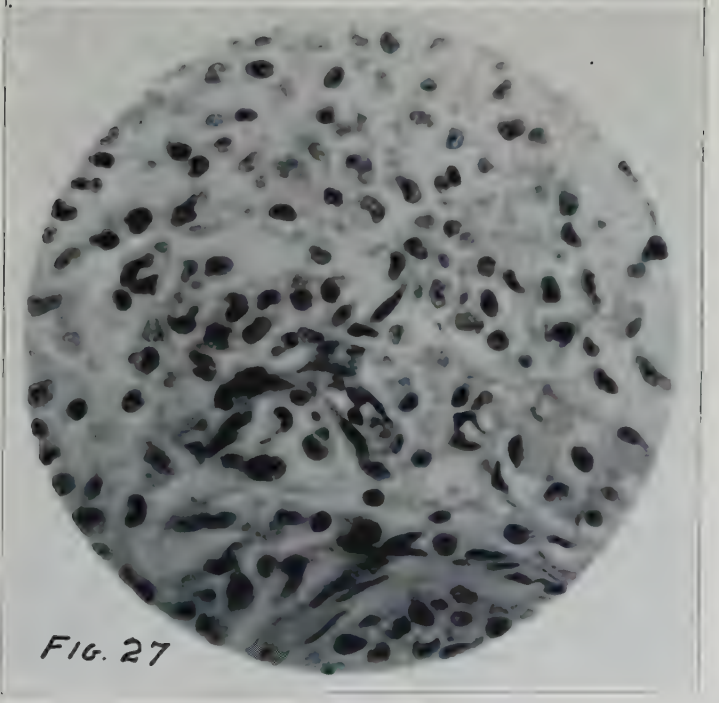


FIG. 27

PLATE XIII

PLATE XIV

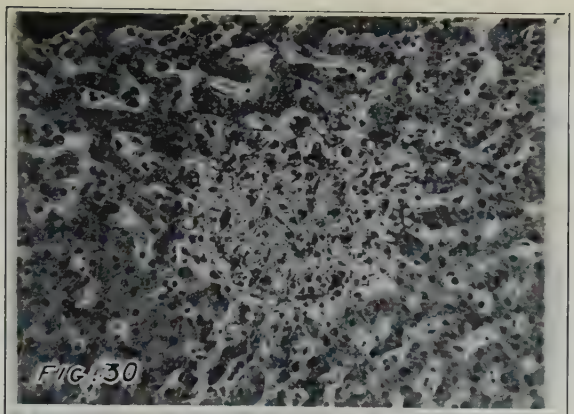


FIG. 30

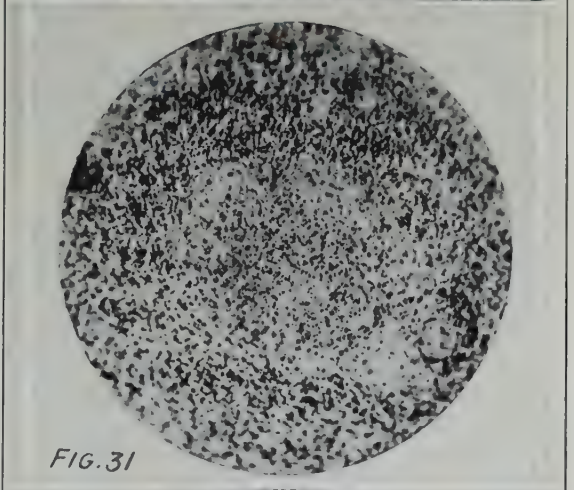


FIG. 31

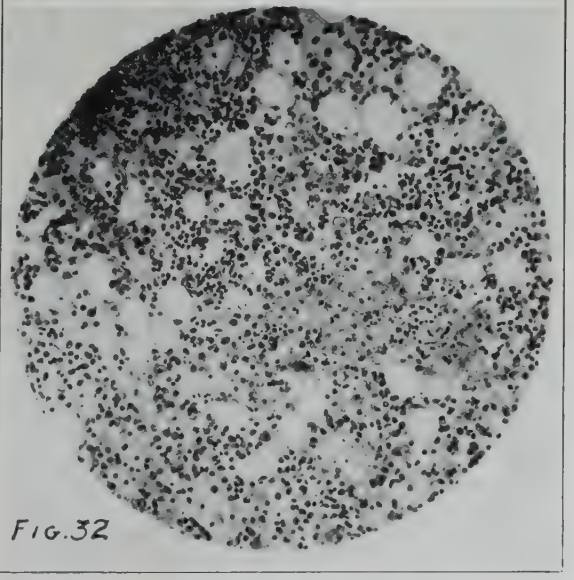


FIG. 32

PLATE XV

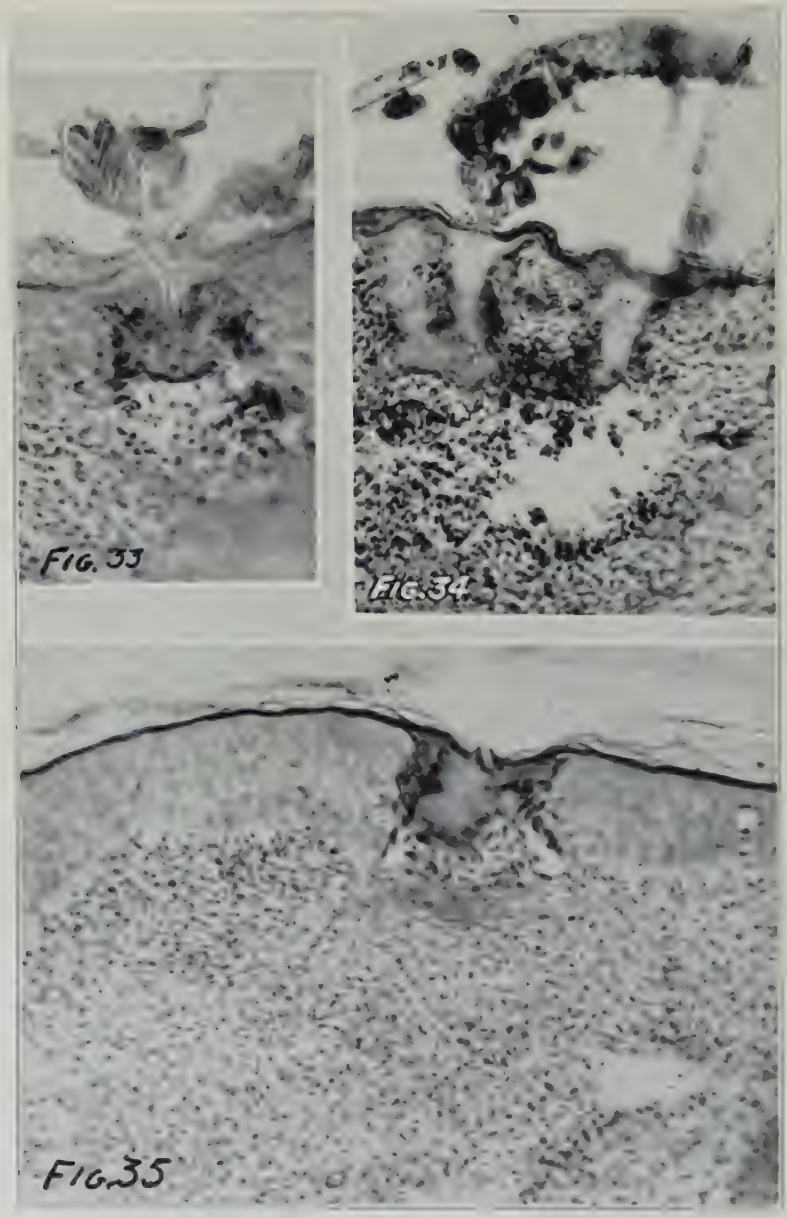
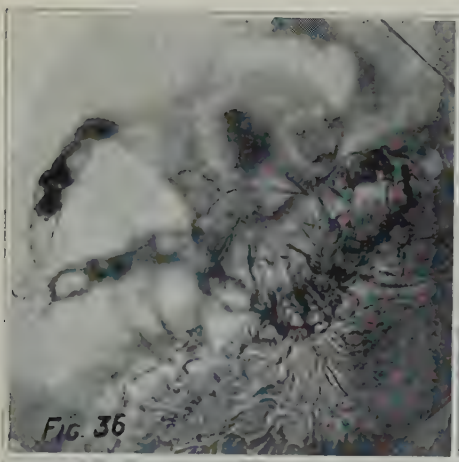
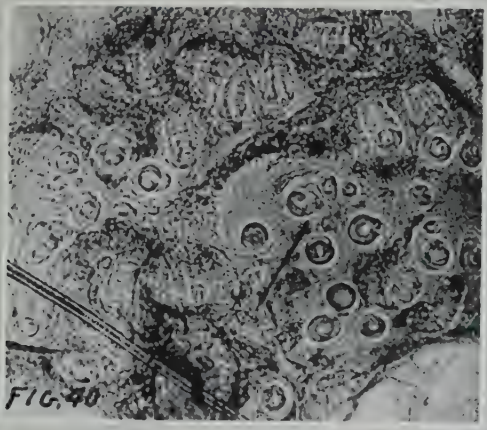
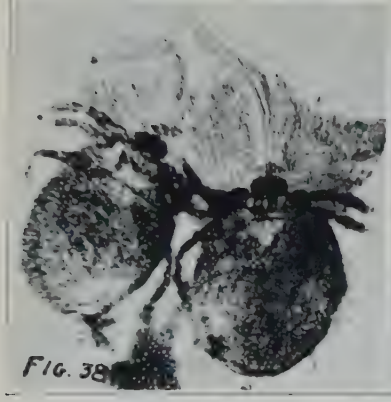


PLATE XVI







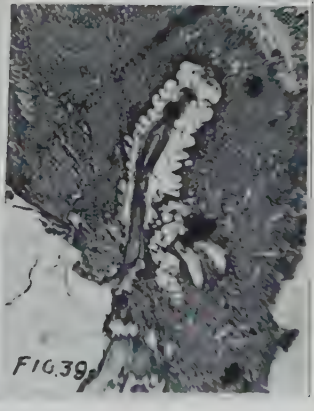


PLATE XVII

FIG. 41

FIG. 42

FIG. 43

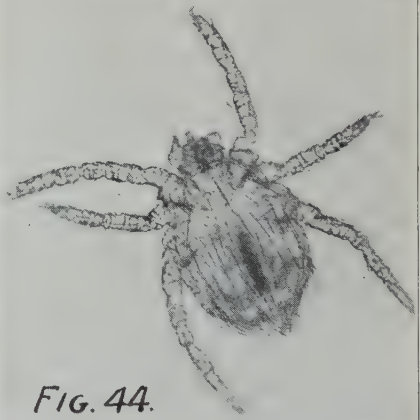


FIG. 44.

FIG. 45

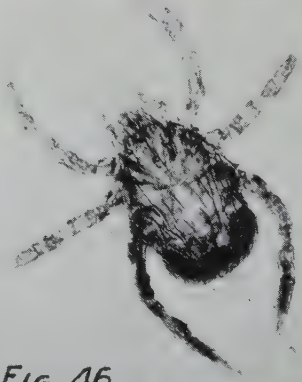


FIG. 46

PLATE XVIII

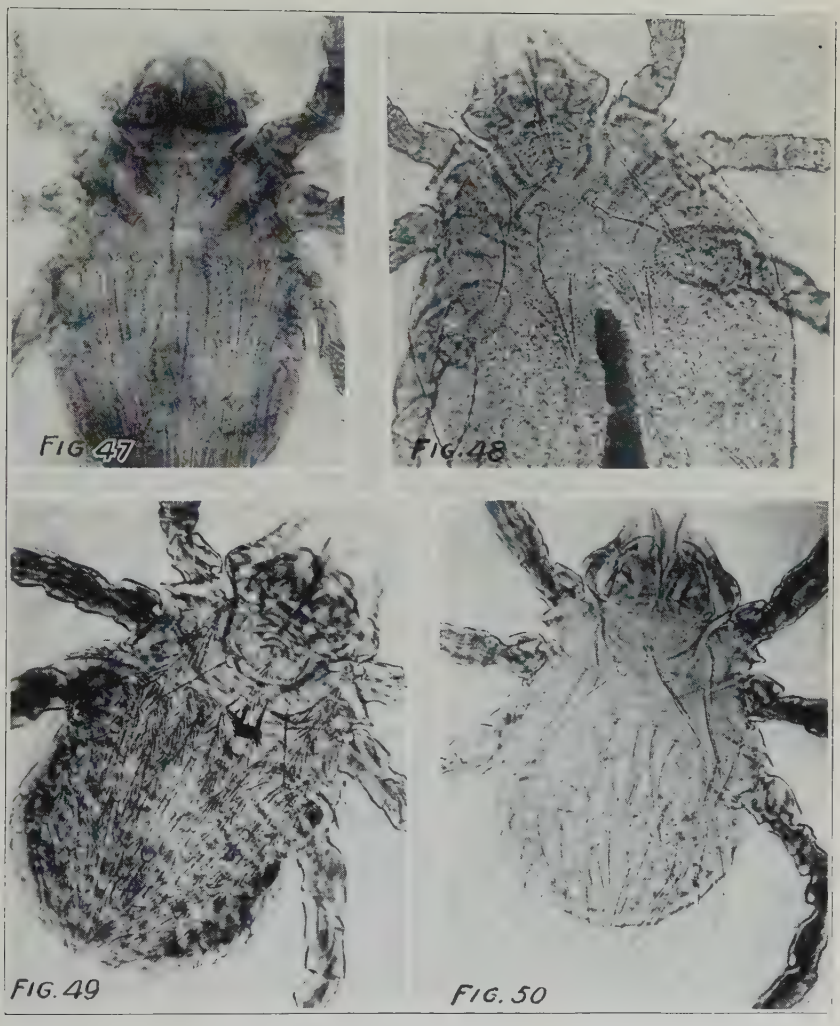


PLATE XIX

FIG. 52

FIG. 51

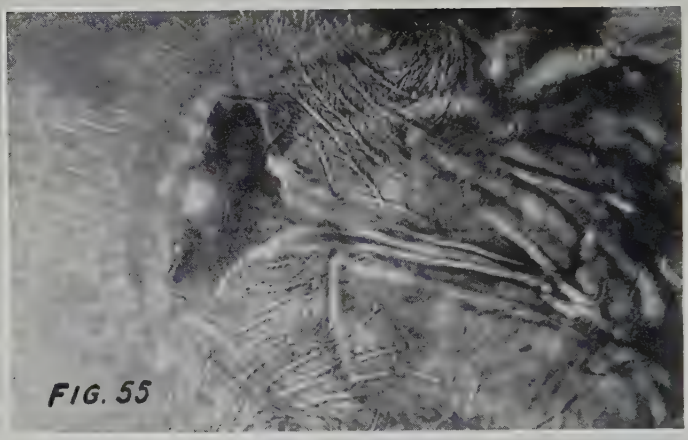


FIG. 55

FIG. 53

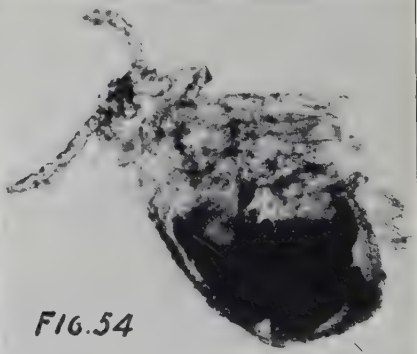


FIG. 54

PLATE XX

FIG. 57

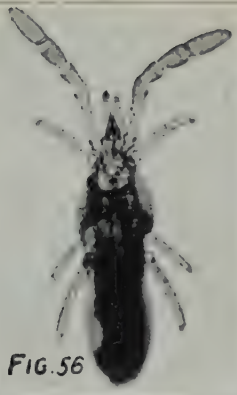


FIG. 56

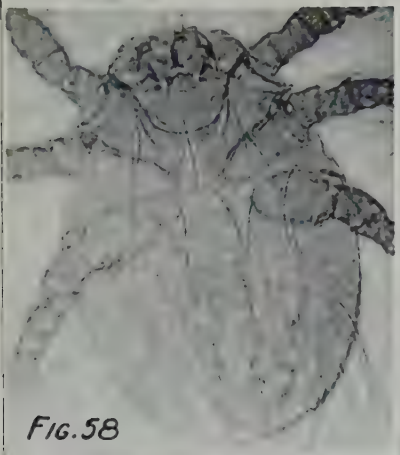


FIG. 58

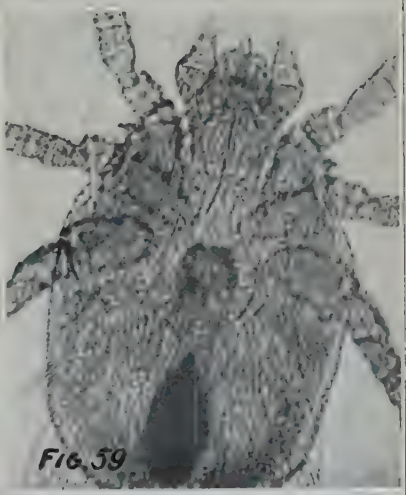


FIG. 59

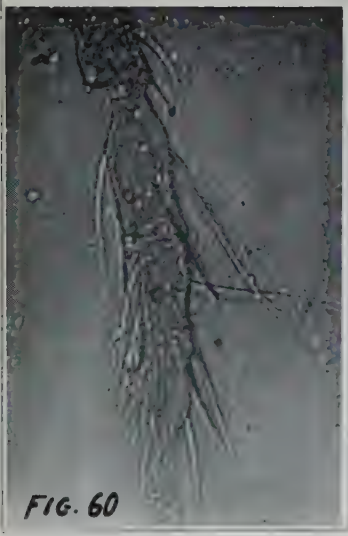


FIG. 60

FIG. 61

PLATE XXI

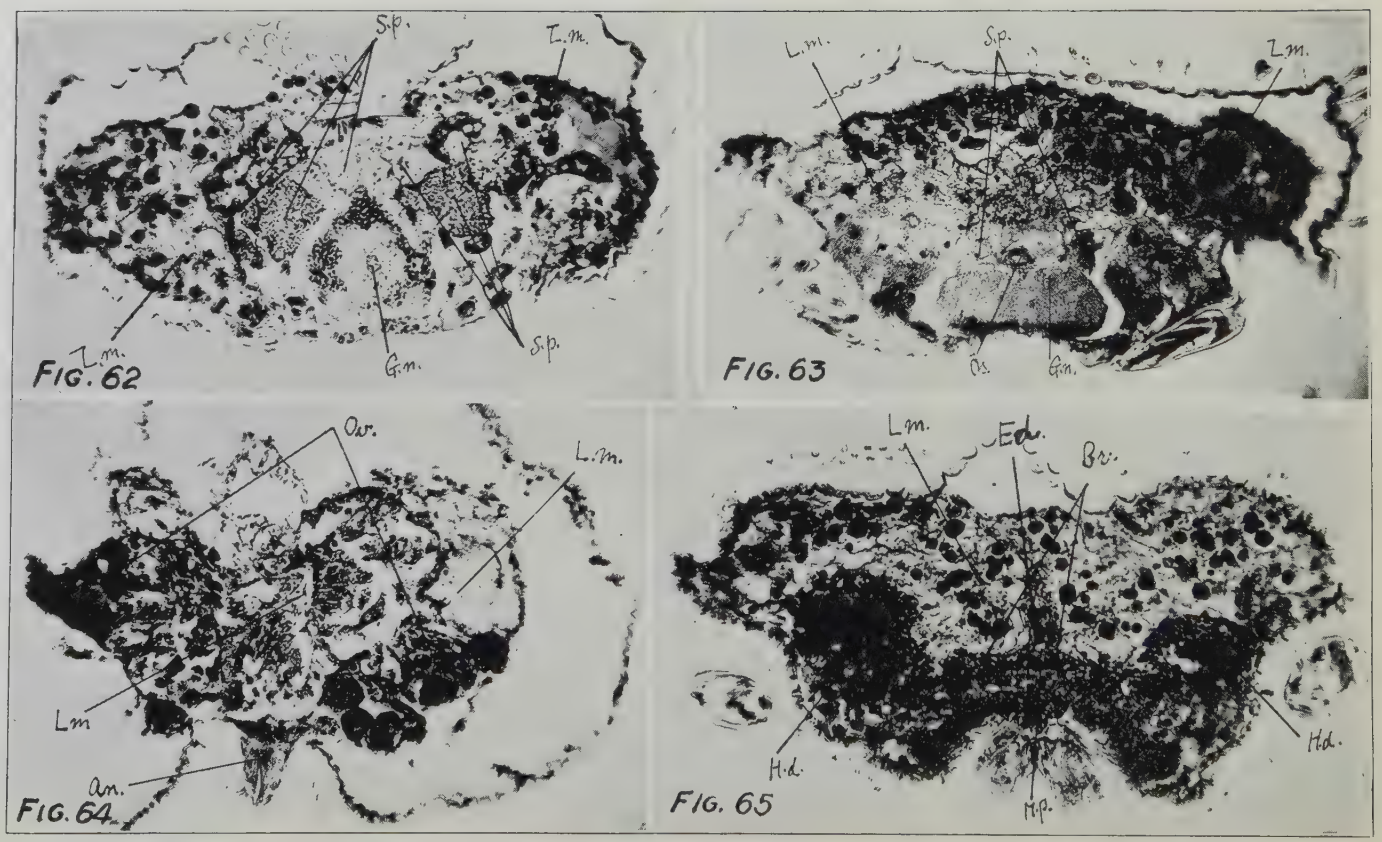

Sp.
L.m.
L.m.
G.m.
Sp.
FIG. 62
L.m.
Sp.
L.m.
Os.
G.m.
FIG. 63
Ov.
L.m.
Lm
An.
FIG. 64
L.m.
Ed.
Br.
H.d.
M.p.
Hd.
FIG. 65

PLATE XXII

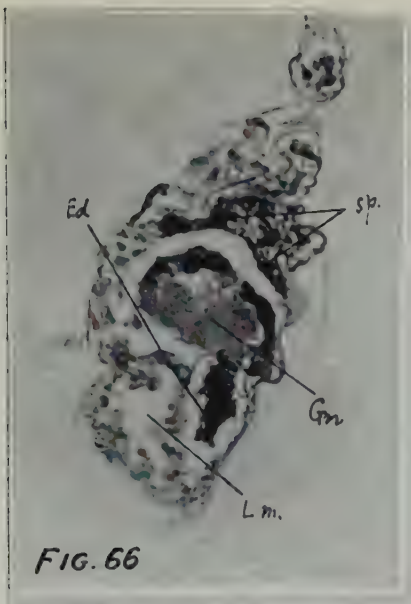


FIG. 66

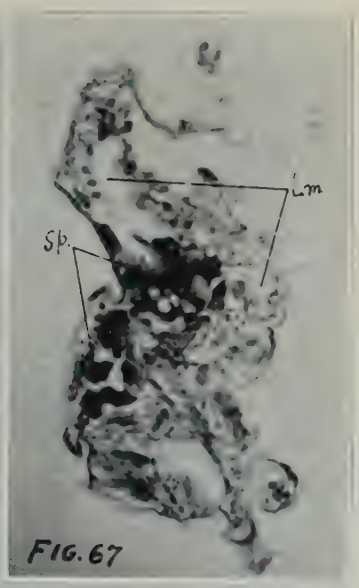


FIG. 67

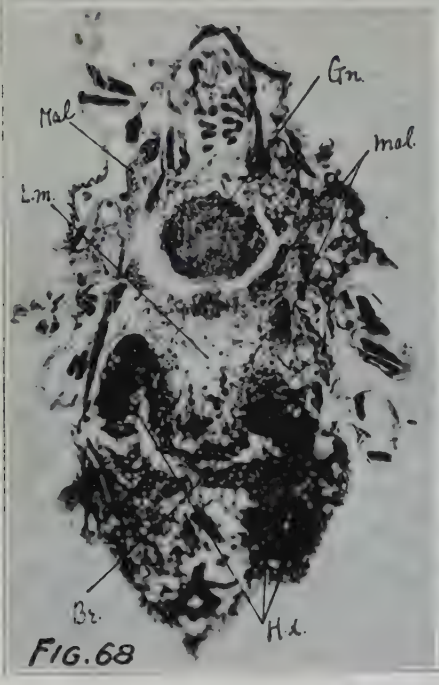


FIG. 68

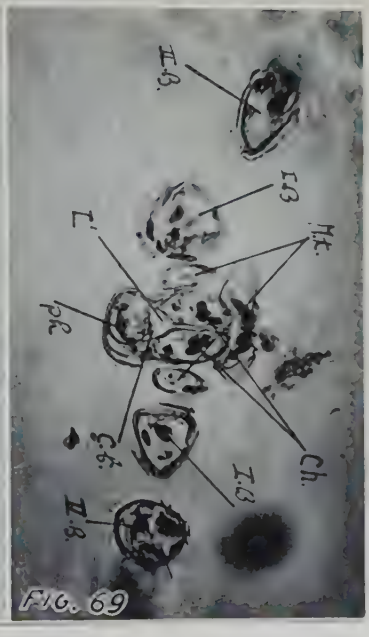


FIG. 69

PLATE XXIII

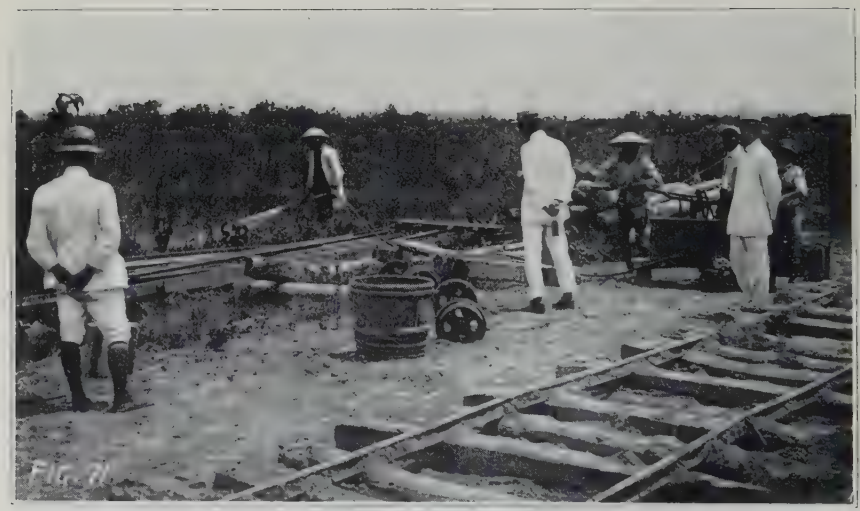


PLATE XXIV

FIG. 72

FIG. 73

FIG. 74

PLATE XXV

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